IDENTIFYING SPECIES SPECIFIC POLYMORPHISM IN RED JUNGLE FOWL *(Gallus gallus murghi)* AND DOMESTICATED CHICKEN *(Gallus gallus domesticus)*

Thesis

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UNDER THE GUIDANCE OF

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INDIA

Dedicated to

My parents

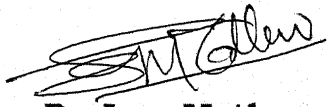
&

My husband

CERTIFICATE

This is to certify that the research work in this thesis entitled "**Identifying species specific polymorphism in red jungle fowl (*Gallus gallus murghi*) and domesticated chicken (*Gallus gallus domesticus*)**" submitted by **Ms. Jaiyashi Gupta** for the award of the **Doctor of Philosophy** in Biotechnology of Bundelkhand University, Jhansi, Uttar Pradesh is an original work carried out by the candidate herself under my supervision and guidance. She has fulfilled all the requirements of the ordinance relating to the award of PhD degree of the University.

It is further certified that **Ms. Jaiyashi Gupta** has put in more than 200 days attendance in Department of Biotechnology, Bundelkhand University, Jhansi as required under the relevant ordinance.



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Jaiyashi Gupta

ABBREVIATIONS

°C	Degree centigrade
s	Second
min	Minute
h	Hour
µl	Microliter
µg	Microgram
ng	Nanogram
mM	Millimole
M	Mole
bp	Base pair
DNA	Dioxy-ribose nucleic acid
RNA	Ribose nucleic acid
dNTP	Dioxyribonucleotide tri phosphate
g	Gram
rpm	Revolution per minute
ml	Milliliter
%	Percent
PCR	Polymerase Chain Reaction
µlM	Micromole
TBE	Tris Boric EDTA
UV	Ultra violet
OD	Optical density
v/v	Volume by volume

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Introduction

Introduction

The evolution of chicken can be conceived as a three step process – evolution and speciation of wild ancestor, domestication of the wild species; and diversification towards development of new breeds. The domestic chicken (*Gallus gallus domesticus*) originated from southwestern Asia and was first introduced into India in about 3000 BC. Chickens are also depicted in Babylonian carvings of about 600 BC and are mentioned by ancient Greek writers, particularly Aristophanes in 400 BC. The wild Red Junglefowl (*Gallus gallus*) is the single most important species to mankind due to the economic and cultural significance to human civilization, being the progenitor of all the modern days chicken. Presently, Indian Poultry industry is the fastest growing component of the primary sector of Indian economy and has a strong standing globally, being third highest

egg producer with more than 50000 million eggs and fifth highest broiler producer with 2.25 million tones of broiler meat. Largely, Indian poultry industry is chicken dominated and the rearing of high yielding varieties under intensive rearing system is the back bone of its present face. Presently, broiler industry is increasing at a growth rate of 10 %, while egg production has a growth rate of about 6 %. With these growth rates, while the egg production is expected to be 61 billion and more than 100 billion by 2010 and 2020 respectively, the respective estimates of broiler production will be 3.6 million tones and 8.6 million tones Singh and Sharma, 2007). In other words, the present day multi-billion dollar poultry industry is based on the wild RJF and may have to depend on it in the future as well.

Under the genus *Gallus*, there are four species of the Jungle fowl i.e. *Gallus varius* (Green jungle fowl), *Gallus lafayettei* (Ceylon jungle fowl), *Gallus sonneratii* (Grey jungle fowl) and *Gallus gallus* (Red jungle fowl). The domestic chicken (*G. gallus domesticus*) has been placed as one of the subspecies of *Gallus gallus* (Red jungle fowl) while other subspecies are i.e. *G. gallus gallus* (Cochin-chinese RJF), *G. gallus bankiva* (Javan RJF), *G. gallus spadicus* (Burmese RJF) and *G. gallus murghi* (India RJF). The Indus valley used to be considered the domestication point of the chicken about 4000 years ago (Zeuner, 1963), however the remains of domesticated chickens from 16 neolithic sites along the yellow river in Northeast China reveals the divergence of about 8000 years between domestic fowl and its ancestor (West and Zhou, 1989). However, despite of the difference in opinion regarding the domestication period and place, red jungle fowl is believed to be the sole ancestor of domestic chicken. However this monophyletic theory of domestic fowl evolution has been questioned several times. Some evidences in support of polyphyletic theory are the presence of silver gene in domestic fowl and *G. sonneratii*, but absence in *G. gallus*, production of fertile progenies from crosses between domestic fowl and other jungle fowls etc. (Lotsby and Kupier, 1924). However majority of reports favors the monophyletic theory of domestic chicken evolution and *G. gallus* as sole progenitor. Fumihito et al. (1994) suggested a strong possibility of a single domestication event

being the *G. g. gallus*, a major or sole contributor on the basis of divergence in non-coding region of mitochondrial DNA. Further, based on nucleotide sequence variation in D loop region, they found no discernible differences among *G. g. spadicus* and *G. g. gallus*, while *G. g. bankiva* showed differences with both. Phylogenetic relationship between domestic fowl and *G. gallus* subspecies revealed that *G. g. gallus* is the real matriarchic origin of all the domestic poultry (Fumihito et al., 1996). These studies have excluded the two other subspecies of red jungle fowl i.e. *G. g. murghi* and *G. g. jabouillei*.

Recently, fears have been expressed that the wild RJF populations may be genetically contaminated leading to an inference that there may not be any pure RJF populations in the wild (Peterson and Brisbin 1998). These observations were based upon examinations of skins collected in the past from various parts of Asia and preserved in various museums in America and Europe. The authors of this paper contend that in the past, the wild RJF populations have hybridised with domestic, feral and/or domestic stock, especially near by the villages causing the introgression of domestic genes into the wild populations. The skins that were examined by Peterson and Brisbin (1998) showed lack of phenotypic traits, which characterise true wild RJF (Morejohn 1968). However, this reports suffers from several controversial issues regarding the true representative of the wild population, especially in India and Myanmar by the samples used in the study. But, the apparent sampling inadequacy, the threat of hybridisation to the RJF in India is real and needs to be addressed urgently. It calls the need of developing the species specific markers, especially DNA markers, which can provide the dependable standradrs for judging the purity of red jungle fowl and to detect any contamination from feral chicken populations.

In the recent past, molecular genetics have provided several powerful tools such as DNA based genetic markers that can exploit the great wealth of polymorphism at DNA level. DNA-based markers can be grouped into clone/sequence based (CSB) markers and fingerprint markers (Dodgson *et al.* 1997). The first category requires the isolation of a

cloned DNA fragment and often determination of some, if not all, of its DNA sequence. The CSB markers include microsatellite (Weber and May, 1989), RFLP, Sequence Tagged Site (STS) and Expressed Sequence Tags (EST), among others. On the other hand, the fingerprint (FP) markers require no prior knowledge of the sequence of the polymorphic region or isolation of a cloned DNA fragments, and they include variable number off tandem repeats (VNTR). These VNTR are often called minisatellites for large loci (Jeffreys *et al.* 1985) and microsatellites for smaller VNTR loci (Ali *et al.*, 1986). Microsatellite markers are the marker of choice detecting polymorphism between the populations ad identifying the species specific markers (Zhou and Lamont, 1999; Wardecka *et al.*, 2004; Nakamura *et al.*, 2006; Rikimaru and Takahashi, 2007). Apart from the polymorphism in genomic DNA, mitochondrial sequence variation was also used in detecting the polymorphism between the populations and evolutionary studies (Fumihito *et al.*, 1994; Fu *et al.*, 2001a,b; Niu *et al.*, 2002; Silva and Rajapaksha, 2005; Oka *et al.*, 2007).

Therefore, the present study was proposed with the following objectives

1. To detect genetic polymorphism between red jungle fowl and domestic chicken breeds using microsatellite markers
2. To detect genetic polymorphism between red jungle fowl and domestic chicken breeds in mitochondrial DNA.
3. To estimate genetic similarity as well as genetic distance among red jungle fowl and domestic chicken breeds.
4. To establish phylogenic relationship among red jungle fowl and domestic chicken breeds

Review of literature

Review of literature

Microsatellite markers

Microsatellite markers are short DNA fragments of usually less than 100 bp and made up of tandem repeats of 2-6 bp fragments, thus also known as short tandem repeats (STR), single sequence repeats (SSR) or single sequence length polymorphism (SSLP). Microsatellites can be analyzed by PCR amplification of a single tandem repeat locus using primers that anneal at its flanking regions. The PCR amplified fragments expressing the size polymorphism are the alleles at the given microsatellite locus (Weber and May, 1989; Litt and Luty, 1989). Major advantages of these markers are that they are randomly distributed, highly polymorphic, non-functional, so not

subjected to selection and locus specific. They are abundant and are co-dominantly inherited, assayable easily by PCR method and suitable for automated analysis

Application of Microsatellite Markers in Genome Analysis

These markers have been used for estimating the genetic similarity among and within the population in the wide array of germplasm such as bee (Paxton *et al.*, 1996), ants (Seppa and Gertsch, 1996), plants (Gupta *et al.*, 1996; Lanham and Brennan, 1998) and animals (Crawford, 1999). In poultry, these markers are markers of choice in linkage analysis or genome mapping (Khatib *et al.*, 1994; Cheng and Crittenden, 1994; Groen *et al.*, 1994; Groenen *et al.*, 2000). Recently, they are also being used for identification of quantitative trait loci (QTL) and evaluation of genetic diversity within and between populations. There are several examples of genetic diversity studies in chicken employing microsatellites.

Estimation of genetic diversity between individuals/ breeds / populations

Crooijmans *et al.* (1996) studied the microsatellite polymorphism in nine highly selected commercial broiler and six highly selected layer lines. The average number of marker alleles was 5.8 over all lines, 5.2 over broiler lines and 3.0 over layer lines. The average number of marker alleles within a line was 2.9, 3.6 and 2.0 for all, broiler, and layer lines, respectively.

Vanhala *et al.* (1998) used nine microsatellite markers in eight lines of different genetic origin; White Leghorns formed one group, two Finnish landraces formed a second group and the Rhodes Island Red together with broilers lines made up a third group. All microsatellite loci were polymorphic and the number of alleles ranged from 4 – 13 per locus and 1 –10 per line. Based on eight microsatellites isolated from a chicken microsatellite DNA enriched library, 10 native Japanese breeds were clustered into three groups, which corresponded, to their known ancestry (Takahashi *et al.*, 1998).

Zhou and Lamont (1999) analysed 42 microsatellite loci in 23 highly inbred chicken lines derived from Leghorn, Jungle Fowl, Fayoumi and Spanish breeds. Line-specific alleles among breeds and lines were detected. The band-sharing (BS) values and the proportion of shared alleles distances (Dps) between sets of MHC-congenic lines ranged from 0.74 to 0.96, and 0.05-0.35, respectively. The BS values between each pair of noncongenic Leghorn lines were 0.32-0.97, and between Leghorn and exotic (Jungle Fowl, Fayoumi and Spanish) breeds were 0.03-0.55. The Dps between Fayoumi lines and other lines were much larger (0.66-1.34) than within Leghorns, and the Jungle Fowl breed had the largest distances with other lines (1.12-5.38). The phylogenetic consensus tree that was constructed grouped these 23 inbred chicken lines into four different clusters.

Ponsuksili *et al.* (1999) measured genetic distances between 12 chicken lines comparing multilocus DNA fingerprints and multiple single locus microsatellite analysis and found that DNA fingerprints and microsatellites provided similar estimates and heterozygosity.

Using 22 microsatellites Wimmers *et al.* (2000) studied the genetic variability of various indigenous chicken populations of India, Nigeria, Bolivia and Tanzania. They observed that all populations showed high levels of heterozygosity for microsatellite markers and a range of 2 –11 alleles per locus were detected.

Kaiser *et al.* (2000) studied the microsatellite polymorphism between and within broiler populations. They observed high polymorphism for microsatellites in broiler populations with average of 2.8 and 2.9 alleles per line per microsatellite in L and C broiler populations respectively.

The DNA pool of 51 diverse chicken breeds, analyzed using microsatellite marker in a European project on chicken biodiversity revealed that Jungle Fowl and unselected breeds and broilers had more and wide heterogeneity as compared to the layers and experimental lines (Weigend and Romanov, 2001).

Romanov and Weigend (2001) studied the genetic variation and genetic distances between various populations of domestic and jungle fowl using microsatellite markers. In total, they genotyped 224 individuals of 20 populations for 14 microsatellite markers covering 11 linkage groups. Of the 14 microsatellite loci, the number of alleles ranged between 2 -21 per locus (mean number: 11.2 per locus). They reported that the Red Jungle Fowl (*Gallus gallus*) formed a separate branch and demonstrated a specific allele distribution when compared with domestic fowl breeds. The second branch comprised commercial layer lines and chicken breeds that were subject to intense selection in the past or had common ancestral breeds with commercial strain. The third group encompassed the German native breed populations.

Long HuoSheng *et al.* (2002) used 53 arbitrary primers to amplify DNA fingerprints in DNA pools representing 11 chicken population including Red Jungle Fowl they reported that the 8 Chinese domestic chicken populations showed extensive genetic diversity with the two introduced populations showing low genetic diversity and genetic differentiation. There was much genetic differentiation with the Red Jungle Fowl.

Hillel *et al.* (2003) assessed the genetic variation within and between 52 populations from a wide range of chicken type. Twenty two dinucleotide microsatellite markers were used to genotype DNA pools of 50 birds from each population (inbred C line) and the most polymorphic population(*Gallus gallus spadiceus*) were, respectively, as follows : number of alleles per locus, per population: 3.5, 1.3, and 5.2; average gene diversity across markers : 0.47, 0.05, and 0.64; and proportion of polymorphic markers. 0.91, 0.25 and 1.0. They observed that these were in good agreement with the breeding history of the population. They reported that unselected populations were more polymorphic than selected breeds such as layers. Thus DNA pools are effective in the preliminary assessment of genetic variation of Populations and markers.

Pandey *et al.* (2003) studied 3 poultry breeds Nicobari, Aseel and Miri of distant regions of the country for genetic diversity analysis. They selected 26 microsatellite loci in such a way that they represented the complete genome and were unlinked. They reported that unbiased heterozygosity ranged from 0.5078 – 0.8825 in the entire data set and mean unbiased heterozygosity over all loci were 0.62, 0.62, and 0.61 for Nicobari, Miri, Aseel poultry, respectively.

Wang DeQian *et al.* (2003) analyzed the genetic polymorphism of 7 microsatellite loci in 12 Chinese native chicken breeds. It was shown that the mean heterozygosities of Luyuan and Chahau chickens were 0.5929 and 0.3514, which were the highest and the lowest among the breed, respectively.

Cheng *et al.* (2003), using five microsatellite markers with high polymorphisms estimated the genetic diversity of seven chicken breeds and found 40 alleles. While most alleles (10) were detected by ADL0136 and the least (5) were detected by ADL0146. The average heterozygosity in the Shouguang chicken was the lowest (0.3327), and that in other breeds was also less than 0.4 . The PIC values ranged from 0.6169 (Shouguang chicken) to 0.7027 (Laiwu Black chicken). UPGMA tree was completed through analysis of DA genetic distance. In the tree, the Rizhao Pockmarked and the Jining Hundred chicken were first grouped together with a bootstrap value of 92%, before they were grouped with the Laiwu Black and the Shouguang chickens. The Anoka Yellow and the Guangxi Yellow chicken were grouped together with a bootstrap value of 80%, but the Luxi Fighting chicken had its own branch.

Li *et al.* (2004) used 20 microsatellite markers to analyse the genetic relationship among four package lines (A, B, C, D) of egg-type chickens introduced in 2001 an 2002. In total, 65 alleles in 20 microsatellite markers were detected. Average allele number for microsatellite markers is 3.250, and average effective allele number for those markers is 2.395. The PIC of

microsatellite markers averaged at 0.454, ranging from 0.102 to 0.729. The heterozygosity of microsatellite markers ranged from 0.108 to 0.765. The heterozygosity of A2001 (0.390) was lowest, and that of D2001 (0.452) highest. The genetic distances among A and B lines were 0.005-0.016, while those among C and D lines were 0.094-0.119. Genetic resemble coefficients among A and B lines were above 0.984, while those among C and D lines around 0.900. The results proved, on the molecular level, that A and B are identical or two lines but much closed in genetic background while C and D are two different lines.

Wardecka *et al.* (2004) determined microsatellite polymorphism in Rhode Island Red (RIR) and Sussex (SX) chickens, divergently selected over six generations for high (H) or low (L) incidence of skeletal defects in embryos (30.7% for H lines, 3.7% for L lines). The polymorphism analysis covered 15 microsatellite markers within four lines (a total of 60 individuals). Eight alleles were identified as specific to H lines and six alleles as specific to L lines. The selection for skeletal malformation appears to have affected the frequency of microsatellite alleles. The experimental material examined constitutes a valuable source for identification of real genes causing skeletal defects

Tu *et al.* (2005) used 30 microsatellite markers to detect the genetic diversity of 8 indigenous chicken breeds in Sichuan and found that 24 of them were highly polymorphic. The mean heterozygosity of 8 chicken breeds was all over 0.5. The highest was the Luning chicken (0.681), and the lowest was the Jiuyuan Dark chicken. The results of the heterozygosity were consistent with that of PIC. UPGMA tree was completed through analysis of DA genetic distances. Emei Dark chicken, Miyi chicken, Luning chicken and Jiuyuan Dark chicken were the first group: Miyi chicken and Luning chicken were grouped firstly, then Emei Dark chicken were grouped with them in shorter time distances, and Jiuyuan Dark chicken were grouped with them at last. Shimiancao Ke chicken Xingwen Silky chicken and Muchuan Silky were the second group: Xingwen Silky chicken and Muchuan Silky were grouped firstly, and then Shimiancao Ke chicken was grouped with them.

Liangshangya Ying chicken had its own branch. The result of UPGM was consistent with the genesis, breeding history, differentiation and location of 8 chicken breeds.

Nakamura *et al.* (2006) used 25 microsatellite markers to identify the polymorphism between 4 strains of Nagoya breed (native to Japan) from other breeds and commercial stocks of chicken. In these strains, 5 of the markers (ABR0015, ABR0257, ABR0417, ABR0495, and ADL0262) had a single allele. Other chicken samples (448) of various breeds and hybrids were analyzed using the same 5 markers. None of these chicken samples had the same allele combination as the Nagoya breed strains. These 5 microsatellite markers provide a practical method to accurately discriminate the Nagoya breed from other chicken breeds.

Qu *et al.* (2006) investigated the current status of Chinese poultry genetic resources. A total of 78 indigenous chicken breeds were surveyed. A total of 2740 individuals were genotyped for 27 microsatellite markers on 13 chromosomes. The number of alleles of the 27 markers ranged from 6 to 51 per locus with a mean of 18.74. Heterozygosity (H) values of the 78 chicken breeds were all more than 0.5. The average H value (0.622) and polymorphism information content (PIC, 0.573) of these breeds suggested that the Chinese indigenous chickens possessed more genetic diversity than that reported in many other countries. The high genetic diversity in Chinese indigenous breeds is in agreement with great phenotypic variation of these breeds. Using Nei's genetic distance and the Neighbor-Joining method, the indigenous Chinese chickens were classified into six categories that were generally consistent with their geographic distributions.

Haunshi and Sharma (2006), genotyped 76 BC_1 (37 from Type A and 39 from Type B) progenies with 10 informative microsatellite markers i.e. MCW 40, MCW 48, LEI 65, LEI 113, ADL 136, ADL 176, ADL 181, ADL 210 and ROS 109. Average number of alleles per locus (marker) was 3.3 and allele size ranged from 96 to 219 bp. The parental genomic proportion from the donor grand

sire to the BC_1 progenies (estimated in terms of the genetic similarity between grand sire and BC_1 progenies ranged from 0.58 to 0.85 with an average of 0.72. The differences were non- significant for BS estimates between both types of BC_1 populations. The overall mean genetic distance between the grand sire and BC_1 progenies was 0.332 ± 0.010.Within-population genetic similarity in type A and type B populations was insignificantly different and overall estimate was 0.611 ± 0.058, while the genetic similarity as well as genetic distance between the type A and Type B was 0.927 ± 0.026 and 0.093 ± 0.038 respectively.

Mwacharo *et al.* (2007) used 30 microsatellite markers recommended by the International Society for Animal Genetics and the Food and Agriculture Organization to determine the extent of genetic differentiation and phylogenetic relationships among indigenous chicken populations sampled in Kenya, Uganda, Ethiopia and Sudan. Genetic differentiation (F(ST)) and chord genetic distances (D(C)) indicated that the indigenous chickens were genetically related but distinct from commercial broiler and egg layer lines. Genetic divergence among the indigenous chickens determined using the Mantel test was significantly influenced ($P < 0.001$) by geographic (reproductive) isolation. Genetic subdivisions were found between the Kenyan/Ugandan chicken populations and Ethiopian/Sudanese chicken populations. The Marsabit chicken population from northern Kenya was the most genetically distinct population within the Kenyan and Ugandan chicken cluster, thus warranting further investigation.

Rikimaru and Takahashi (2007) used 37 microsatellite markers on the Z chromosome to genotype the 555 individuals from Hinai-jidori chicken to discriminate it from other chickens. Fourteen of the marker loci (ABR1003, ADL0250, ABR0241, ABR0311, ABR1004, ABR1013, ABR0633, ABR1005, ABR0089, ABR1007, ABR1001, ABR1009, ABR1010, and ABR1011) were fixed in the Hinai-dori breed. Absence of these alleles on 14 loci from the Hinai-dori breed in meat samples suggested that the samples under test were not the Hinai-jidori chicken.

Muchadeyi *et al.* (2007) investigated the population structure of village chickens found in the five agro-ecological zones of Zimbabwe. Twenty-nine microsatellites were genotyped for chickens randomly selected from 13 populations, including the five eco-zones of Zimbabwe (n = 238), Malawi (n = 60), Sudan (n = 48) and six purebred lines (n = 180). A total of 280 alleles were observed in the 13 populations. Forty-eight of these alleles were unique to the Zimbabwe chicken ecotypes. The average number (± SD) of alleles/locus was 9.7 ± 5.10. The overall heterozygote deficiency in the Zimbabwe chickens (F(IT) ± SE) was 0.08 ± 0.01, over 90% of which was due to within-ecotype deficit (F(IS)). Small Nei's standard genetic distances ranging from 0.02 to 0.05 were observed between Zimbabwe ecotypes compared with an average of 0.6 between purebred lines. The structure software program was used to cluster individuals to 2 </= K </= 7 assumed clusters. The most probable clustering was found at K = 6. Ninety-seven of 100 structure runs were identical, in which Malawi, Sudan and purebred lines split out as independent clusters and the five Zimbabwe ecotypes clustered into one population. The within-ecotype marker-estimated kinships (mean = 0.13) differed only slightly from the between-ecotype estimates. Results from this study lead to a rejection of the hypothesis that village chickens are substructured across agro-ecological zones but indicated high genetic diversity within the Zimbabwe chicken population

Tadano *et al.* (2007) evaluated the genetic diversity and relationships of 9 native Japanese long-tailed chicken breeds (Shoukoku, Koeyoshi, Kurokashiwa, Minohiki, Ohiki, Onagadori, Satsumadori, Toumaru, and Toutenkou) together with 2 commercial breeds (White Leghorn and White Plymouth Rock), using 40 polymorphic microsatellite markers covering 23 linkage groups. The 8 breeds mentioned, except for Shoukoku and 2 commercial breeds, were believed to be descendants derived from crossings of the ancestor of Shoukoku and some other breeds. Three to 14 alleles per locus were detected across all the breeds. The mean number of alleles per locus, the mean unbiased expected heterozygosity, and the mean

polymorphic information content ranged from 2.60 (Minohiki) to 4.07 (Shoukoku), from 0.293 (Koeyoshi) to 0.545 (Satsumadori), and from 0.250 (Koeyoshi) to 0.478 (Satsumadori), respectively. The mean fixation coefficient of subpopulation within the total population of 9 Japanese long-tailed breeds showed that approximately 38% of the genetic variation was caused by breed differences and 62% was due to differences among individuals. Toumaru had the largest number of breed-specific alleles with relatively high (>20%) frequency. In the phylogenetic tree of 11 breeds constructed by the neighbor-joining method from modified Cavalli-Sforza chord genetic distance measure, White Leghorn and White Plymouth Rock clustered together apart from the Japanese breeds. Among the Japanese long-tailed breeds, Toumaru, Kurokashiwa, and Koeyoshi showed relatively far distance from the other breeds. The Ohiki, Onagadori, Shoukoku, and Toutenkou were grouped into the same branch. Minohiki and Satsumadori were also clustered together. Kurokashiwa was not genetically close to Shoukoku, differing from a traditional hypothsis.

Shahbazi S (2007) characterized five native chicken populations located in the northwestern (West Azerbaijan), northern (Mazandaran), central (Isfahan, Yazd), and southern (Fars) provinces of Iran using five polymorphic microsatellite markers. The number of alleles ranged from three to six per microsatellite locus. All populations were characterized by a high degree of genetic diversity, with the lowest heterozygosity found in the Isfahan population (62%) and the greatest in the populations from West Azerbaijan and Mazandaran (79%). The largest Nei's unbiased genetic distance was found between the Isfahan and Fars populations (0.696) and the smallest between the Mazandaran and Yazd populations (0.097). The Isfahan population was found to be the most genetically distant among all populations studied. These results serve as an initial step in the plan for genetic characterization and conservation of Iranian native chickens.

Tomar et al (2007) detected genetic polymorphism between red jungle fowl (RJF) and domestic chicken i.e. Aseel (AS), Red Cornish (RC),

White Leghorn (WL) using 5 microsatellite markers i.e. ADL 237, LEI 65, LEI 113, MCW 156 and ROS 54. The number of alleles per locus amplified ranged from 3 (LEI 113) to 6 alleles (ADL 237 and LEI 65). In general, the size of alleles ranged from 96 bp to 290 bp and the majority of alleles were in the size range of 110 bp to 2756 bp. The mean within-breed genetic similarity in AS, RC, WL and RJF populations were 0.646, 0.659, 0.759 and 0.693, respectively. Between breed genetic similarity estimates pooled over different micro satellite markers ranged from 0.421 between RJF and WL to 0.492 between RJF and RC. In general, RJF showed lower genetic similarity with all the other three breeds in comparison to other combinations and among three breeds, it showed maximum genetic similarity with Aseel. Similarly, the between breed genetic distances estimates pooled over different microsatellite markers ranged from 0.256 (AS and RC) to 0.856 (RJF & WL).

Mitochondrial DNA (mtDNA)

In 1949, Boris Ephrusi suggested that mitochondria have their own genome and several years later, in the mid 1970, mitochondria were found to contain DNA. The DNA found in the mitochondrion is called mitochondrial DNA (mtDNA). They are also known as extra-nuclear genes or cytoplasmic genes or organellar genes, extra-chromosomal genes or non Mendelian gene. Since the cytoplasm is inherited from the mother, the inheritance of such cytoplasmic factor is strictly maternal. Recently, the application of modern molecular biological technique has led to rapid advances in knowledge about mitochondrial genome.

The avian mitochondrial DNA is a double helical circular DNA contains 37 genes (13 proteins,22 tRNAs and 2 rRNAs). Desjardins and Morais (1990) studied the complete mitochondrial (mt) genome sequences of WLH chicken (*Gallus gallus domesticus*). Sequence analysis revealed that the WLH chicken mt genome is 16.775 bp and the genome encodes the same set of genes (13 proteins, 2 rRNAs and 22 tRNAs) as do other vertebrate mt DNAs and is organized in a very similar economical fashion. There are very few inter-genic

nucleotides and several instances of overlaps between proteins as tRNA genes. The protein genes are highly similar to their mammalian and amphibian counterparts and are translated according to the same variant genetic code. Despite these highly conserved features, the chicken mt genome displays two distinctive characteristics. First it exhibits a novel gene order, the contiguous $tRNA^{Glu}$ and ND6 genes are located immediately adjacent to the displacement loop region of the molecule, just ahead of the contiguous $tRNA^{Pro}$, $tRNA^{Thr}$ and Cyt b gene which border the d-loop region in other vertebrate mt genomes. This unusual gene order is conserved among the galliform birds, second, a light stand replication origin, equivalent to the conserved sequence found between mt tRNA Cys and tRNA-Asn genes in all vertebrate mt genome sequences thus far, is absent in the chicken genome. These observations indicate that galliform mt genomes departed from their mammalian and amphibian counterparts during the course of evolution of vertebrate species.

Mitochondrial DNA is effectively a short cut or an index to the workings of the nucleus and serves as an indicator of the nuclear DNA, making it possible to monitor changes in the nuclear genome on an ongoing basis. The rapid rate of evolution of mt DNA together with the maternal inheritance, smaller size, no recombination and large number of mitochondria per cell which permit mtDNA extraction from minute and degraded samples and all these qualities have made the mtDNA a useful tool for the study of evolution of closely related species (Loftus *et al.*, 1994; Bradley *et al.*, 1996 ; Manceau *et al.*, 1999; and Luikart *et at.*, 2001, Ingman *et al*, 2001). Mitochondrial DNA plays an important role in investigation population structure and phylogenetic relationship of different animals as well as different avian species which has greater role in conservation biology and species identification.

mtDNA polymorphism and its application in phylogeny in chicken and related species

Fumihito *et al.*, (1994) studied the sequences of the first 400 bases of non-coding region of control region. Unlike its close ally *Gallus varius* (green jungle fowl), the red jungle fowl (*Gallus gallus*) is a genetically very diverse species. The red jungle fowl from Thailand (*G. g. gallus* and *G. g. spadiceus*) showed divergence of 7 % with that from Indonesian island of Java (*G. g. Bankiva*). Furthermore, the divergence increased to 27.83 % if each transversion is regarded as an equivalent of 10 transitions. On the other hand, a mere 0.5-3.0% difference (all transitions) were observed between the various domestic breeds of the chicken and two *G. g. gallus* of Thailand, indicating a single domestication event in the area inhabited by this subspecies of the red jungle fowl as the origin of all domestic breeds. Only transitions separated six diverse domesticated breeds.

Fumihito *et al.*, (1995) compared the mitochondrial control-region DNA base sequences of 16 avian species belonging to the subfamily Phasianinae and found that 12 species of phasianine birds belonged to three distinct branches. The first branch was made exclusively of members of the genus *Gallus*, while the second branch was made of pheasants of the genera *Phasianus*, *Chrysolophus*, and *Syrmaticus*. Gallopheasants of the genus *Lophura* were distant cousins to these pheasants. The great argus (*Argusianus argus*) and pea-fowls of the genus *Pavo* constituted the third branch.

Fumihito *et al.*, (1996) sequenced the D-loop regions for a total of 21 birds, of which 12 samples belong to red jungle fowl (*Gallus gallus*) comprising three subspecies (*six G. g. gallus*, three *G. g. spadiceus*, and three *G. g. bankiva*) and nine represent diverse domestic breeds (*G. g. domesticus*). They also sequenced four green jungle fowl (*Gallus varius*), two Lafayette's jungle fowl (*Gallus lafayettei*), and one grey jungle fowl (*Gallus sonneratii*). They found that a continental population of *G. g. gallus* was the real matriarchic origin of all the domestic poultries examined in this study. It is also of particular interest that there were no discernible differences among *G.*

gallus subspecies; *G. g. bankiva* was a notable exception. This was because *G. g. spadiceus* and a continental population of *G. g. gallus* formed a single cluster in the phylogenetic tree. The G. g. bankiva, on the other hand, was a distinct entity, thus deserving its subspecies status. It implies that a continental population of *G. g. gallus* sufficed as the monophyletic ancestor of all domestic breeds.

Munechika *et al.,* (1997) analyzed the restriction endonuclease cleavage patterns of mtDNA. Sequence divergence between *Gallus gallus* and *G. sonnerati, G. varius* and *G. lafayattei* was 0.9. 10.5 and 12.6 % respectively. Assuming that the rate of evolution of mt DNA is 3 % per million years, It was estimated that the divergence of mtDNA of *G. gallus* from that of *G. sonnerati, G. varius* and *G. lafayattei* occurred 0.3, 3.5 and 4.5 million years ago, respectively.

Fu *et al.,* (2001a) sequenced 539 bases of mitochondrial DNA D-loop region of six domestic chicken breeds (30 individuals) and compared to that of red jungle fowl, grey jungle fowl, green jungle fowl and Lafayette's jungle fowl, and the phylogenetic trees for the chickens constructed based on the D-loop sequences. The results indicated that the four species of genus Gallus had great differences between each other, the *G. g. domesticus* was the next of kin to red jungle fowl in Thailand and its adjacent regions, and near of kin to red junglefowl in Indonesian, Lafayette's jungle fowl, grey jungle fowl and green jungle fowl one by one in proper order, suggesting that the red jungle fowl in Thailand and its neighbour areas sufficed as the matriarchic ancestor of Chinese domestic fowls. It was also discovered that the two subspecies of Thailand i.e. G. g. gallus and G. g. spadiceus should belong to one subspecies because of their far lower differentiation compared to that among the domestic breeds.

Fu *et al.,* (2001b) sequenced 539 bases of mitochondrial DNA D-loop region of five native chicken breeds of Zhejiang province and the white leghorn chicken and the phylogenetic trees of the chicken breeds were

constructed. The results showed that 24 variation sites i.e. 4.45% sequence divergence were detected among the 30 DNA sequences, and the six breeds belonged to two different maternal lineage, one included Xianju chicken and White Leghorn chicken which had the same maternal origin, the other included Lingkun chicken, Baiyiner chicken, Wugu chicken and Xiaoshan chicken which had the same matriarchic ancestor. Among the latter lineage, Lingkun chicken, Baiyiner chicken and Wugu chicken had a closer relationship to each other than to Xiaoshan chicken.

Niu *et al.*, (2002) sequenced first 539 bases of mitochondrial DNA D-loop region of six Chinese native chicken breeds (*G. g. domesticus*) were sequenced and compared to those of the red jungle fowl (*Gallus gallus*), the gray jungle fowl (*Gallus sonneratii*), the green jungle fowl (*Gallus varius*) and Lafayette's jungle fowl (*Gallus lafayettei*), and the phylogenetic trees for the chickens were constructed based on the D-loop sequences. The results showed that the four species of the genus Gallus had great differences among each other the G. g. domesticus was closest to the red jungle fowl in Thailand and its adjacent regions, suggesting the Chinese domestic fowl probably originated from the red junglefowl in these regions. The two subspecies of Thailand, *G. g. gallus* and *G. g. spadiceus*, should belong to one subspecies because of their resemblance. In the case of native breeds, there existed a great difference between the egg breeds and general purpose breeds, which suggested different maternal origins of the two types.

Shen *et al.*, (2002) used complete cytochrome b (cyt b) gene (1140 bp) nucleotide sequences to investigate characteristics of the genetic constitution of Chunky broiler chickens, and these were compared with the Hy-Line and WL-GM (Garber) line of White Leghorn, the GSP line of Fayoumi, the BM-C line of Black Minorca (egg-chickens), and an outgroup of wild-origin Japanese quail. A high genetic difference (five haplotypes) was observed at the cytochrome b region in the Chunky broiler in contrast to the high homologies observed among the other chicken breeds (egg-purpose). Chunky broilers can be distinguished from the other breeds (White Leghorn,

Fayoumi, and Black Minorca) at positions 552 and 779. The molecular phylogenetic tree exhibited genetic differences within Chunky broilers, and between Chunky broilers and the other three chicken breeds. As a result, some chicken strains or breeds apparently different from the other egg-chickens may have contributed to the Chunky broiler formation. Artificial selection may be one of the biggest factors causing nucleotide diversity in the chicken breeds.

Liu *et al.* (2004) investigated the genetic variability of 544 bp mt DNA hyper variable HVSI) in a total of 48 birds belonging to 12 Chinese native chicken breeds and identified 16 from 35 polymorphic nucleotide sites, which accounted for 6.4 %. Diversity analysis of the haplotypes showed that Tibetan, Langshan and Henan cockfight chicken had only one haplotypes, while ancient haplotypes existed in Taihe silkie and Chahua chicken. Phylogenetic analysis of the haplotypes suggested that Chinese native chicken breeds shared the same maternal lineage, regardless of their external features and ecological types. Both divergent and phylogenetic analysis of the haplotypes indicated the close genetic relationship between the Chinese native chicken breeds and *Gallus gallus* and G. *gallus spadiceus* from different areas, suggesting that G. *g. gallus* and G. *g. spadiceus* were the original ancestors of Chinese native chicken breeds.

Wada *et al.,* (2004) sequenced the whole mt DNA of the silkie fowl (G. *g. domesticus*). The length of silkie mt DNA was found to be 16,784 bp. The similarity between silkie and white leghorn was found to be 99.77% (39 nucleotide differences in 16, 784 bp)

Silva and Rajapaksha (2005) investigated the origin of native fowl in Sri Lanka and to establish genetic relationships between Ceylon Jungle fowl; eleven types of native chicken from Sri Lanka; and two exotic chicken breeds (Cornish and Rhode Island Red) Randomly Amplified Polymorphic DNA (RAPD) analysis with 16 random primers. Genetic similarity indices ranged from 0.5 to 1.1 in average genetic distance scale, indicating a broad genetic

base in the samples studied. Cluster analysis revealed a clear separation of Ceylon Jungle Fowl from all other types studied, indicating that there was early separation and divergent evolution of Ceylon Jungle Fowl from all the domestic races studied

Nishibori *et al.*, (2005) determined sequences of whole mitochondrial DNA (mtDNA) and two segments of the nuclear genome (intron 9 of ornithine carbamoyltransferase, and four chicken repeat 1 elements) for the species in the genus Gallus. The phylogenetic analyses based on mtDNA sequences revealed that two grey jungle fowls (GyJF) were clustered in a clade with RJFs and chicken, and that one GyJF was located in a remote position close to Ceylon junglefowl (CJF). The analyses based on the nuclear sequences revealed that alleles of GyJFs were alternatively clustered with those of CJF and with those of RJFs and chicken. Alternative clustering of RJF and chicken alleles were also observed. These findings taken together strongly indicate that inter-species hybridizations have occurred between GyJF and RJF/chicken and between GyJF and CJF.

Liu *et al.*, (2006a) assessed the origin of chicken and phylogeographic history by analyzing the mitochondrial DNA hyper variable segment I (HVS-I) for 834 domestic chickens (*G. g. domesticus*) across Eurasia as well as 66 wild red jungle fowls (*Gallus gallus*) from Southeast Asia and China. Phylogenetic analyses revealed nine highly divergent mtDNA clades (A–I) in which seven clades contained both the red jungle fowls and domestic chickens. There was no breed-specific clade in the chickens. The clades A, B, and E are distributed ubiquitously in Eurasia, while the other clades were restricted to South and Southeast Asia. Clade C was mainly distributed in Japan and Southeast China, while clades F and G were exclusive to Yunnan, China. The geographic distribution of clade D was closely related to the distribution of the pastime of cock fighting. Statistical tests detect population expansion within each subclade. These distinct distribution patterns and expansion signatures suggest that different clades may originate from different regions, such as Yunnan, South and Southwest China and/or surrounding areas (i.e.,

Vietnam, Burma, and Thailand), and the Indian subcontinent, respectively, which support the theory of multiple origins in South and Southeast Asia.

Liu *et al.*, (2006b) compared the available mtDNA control region sequences in Chinese and Japanese gamecocks to test the recently proposed hypothesis behind the dual origin of the Japanese cockfighting culture (from China and Southeast Asia independently). They assigned gamecock mtDNAs to different matrilineal components (or phylogenetic clades) that emerged from the phylogenetic tree and network profile, and compared the frequency differences between Chinese and Japanese gamecocks. Among the six clades (A-F) identified, Japanese gamecocks were most frequently found in clades C and D (74%, 32/43), whereas more than half of the Chinese gamecock samples (69%, 35/51) were grouped in clades A and B. Haplotypes in Japanese gamecocks assigned to clades A, B, and E were either shared with those of the Chinese samples or differed from the close Chinese types by no more than a three-mutation distance. This genetic pattern is in accordance with the proposed dual origin of Japanese gamecocks but has left room for single origin of Japanese gamecocks from China. The genetic structure of gamecocks in China and Japan might also be influenced by subsequent breed selection and conservation after the initial gamecock introduction.

Guan *et al.*, (2007) used experimental and in-silico tools to identify nucleotide variants in the mtGenome, including the coding and non-coding (D-loop) regions. The distribution of the experimentally identified mitochondrial DNA variants in meat- (broilers) and egg-type (White Leghorn) chickens was also assessed. A total of 113 single-nucleotide polymorphisms (SNPs) were identified. The in-silico analysis revealed a total of 91 SNPs, with 70 in the coding region and 21 in the non-coding region. Of the 41 experimentally identified SNPs, 27 were in the D-loop. Together, the experimentally identified SNPs in the non-coding region formed 11 haplotypes, whereas the 14 SNPs in the coding region formed 6. Though, 9 of the D-loop region haplotypes were observed only in broilers, 3 of the 6

haplotypes from the coding region occurred at a significantly higher frequency in broilers.

Oka *et al.*, (2007) analysed the mitochondrial DNA D-loop region of Japanese native chickens to clarify their phylogenetic relationships, possible maternal origin and routes of introduction into Japan. Seven haplogroups (Types A-G) were identified. Types A-C were observed in Jidori, Shokoku and related breeds. However, Type C was absent in Shokoku, which was introduced from China, while most Indonesian native chickens were included in the Type C haplogroup. Types D-G were observed in Shamo and related breeds. Type E had a close genetic relationship with Chinese native chickens. Our results indicate that some breeds were not introduced into Japan as suggested in conventional literature, based on low nucleotide diversity of certain chicken breeds. Sequences originating from China and Korea could be clearly distinguished from those originating from Southeast Asia. In each group, domestic chickens were divided into the Jidori-Shokoku and Shamo groups. These results indicate that Chinese and Korean chickens were derived from Southeast Asia. Following the domestication of red jungle fowl, a non-game type chicken was developed, and it spread to China. A game type chicken was developed in each area. Both non-game and game chickens formed the foundation of Japanese native chickens.

Materials & methods

Materials & methods

I. Microsatellite polymorphism between red jungle fowl and domestic chicken breeds

A. Resource populations & extraction of genomic DNA

A total of five population namely red jungle fowl, White Leghorn, Kadaknath, Aseel and Red Cornish were used. A total of 20 individuals from each population were included in the study. About 0.5 ml of blood was collected from Jugular vein in 1.5ml-eppendorf tube containing EDTA from each individual of all the 5 populations. All the blood samples were stored at –20 °C till further processing. The high molecular weight genomic DNA was isolated using the following simple method. To 50 µl of blood, 700 µl of lysis

buffer (10 mM Tris. HCl, 100 mM NaCl, 1 mM EDTA, pH: 8.0 and 0.5% SDS) containing 60 µg of proteinase K (20 mg/ml) was added. The mixture was vigorously vertexed and incubated at 37 °C for 10-12 hours with gentle shaking. The DNA was purified by extracting with equal volume of phenol, phenol-chloroform and chloroform-isoamylalcohol (24:1). The genomic DNA was precipitated by adding 0.1 volume of 3 M sodium acetate and two volumes of ice chilled ethanol and centrifuged for 5 minutes at 14000 g. DNA pellet was then washed with 70% ethanol, air-dried and subsequently resuspended in TE buffer (10 mM Tris. HCl, 1mM EDTA).

After the complete dissolution of DNA, its optical density at 260 and 280 nm was determined by UV spectrophotometry. The purity of DNA was checked by taking the ratio of optical density at 260 and 280 nm. The DNA samples having O.D ratio between 1.7 and 1.9 have been used for the study.

It is known the one O.D. unit at 260 nm equals 50 µg/ml of pure, double stranded DNA. Therefore, the concentration of DNA samples was calculated by the following formula

Concentration (µg/ml) = O.D. at 260 nm x Dilution ratio x 50

The quality of genomic DNA was also examined by horizontal electrophoresis of DNA samples on 0.8 % agarose gel. Loading samples were prepared by adding approx. 1.0 - 2.0 µl of DNA, 2 µl of 6X Bromophenol blue and 5 µl of distilled water. Electrophoresis was performed at 2 V/cm (Max. 5 V/cm of gel) for 1 to 2 hrs. Finally the gel was examined under UV light. The good quality DNA samples having intact DNA bands without any smearing were selected for further analysis. From each population, 15 individuals, having the ratio of OD260 and OD 280 were selected and used for further analysis. The genomic DNA from each individual was diluted to the concentration of 25-30 ng /µl.

B. Microsatellite Marker Analysis

Selection of Markers

A set of 22 di-nucleotide microsatellite markers i.e. (CA)n used by Hillel et al (2003) in Poultry Biodiversity programme were used. These markers are universally distributed as possible throughout the chicken genome. The genomic position and the 5'-3' sequence of forward and reverse primers for each marker is shown in Table 1. The primers for each microsatellite locus were synthesiszed commercially.

PCR Amplification

Initially the amplification conditions, reported in literature for these markers were tested. In the cases, where these conditions did not produce optimum amplification, the same were standardized by varying various parameters. Finally the following amplification conditions were used.

Amplification reactions were carried out in a final volume of 15 µl reaction mixtures in 0.2 ml thin wall PCR tubes. Each PCR tube containing 25-50 ng genomic DNA, 1.5 mM Mgcl2. 50 mM Kcl, 10 mM Tris-Hcl, pH=8.8, 0.1% Triton X-100, 0.01% gelatin, 200 µM of each dNTP (dATP, dGTP, dCTP and dTTP), 0.5 unit of Taq DNA polymerase enzyme and 10 pico mole of each forward and reverse primer.

The amplification was carried out in a thermocycler (Eppendorf-Germany). Protocol for PCR reaction consisted of an initial denaturation at 94 0C for 5 min. Followed by 30 cycles of PCR, each cycle consisting of 30 s at 94 0C, 45 s at 55 0C and 1.45 min at 72 0C, and followed by a final extension step of 10 min at 72 0C. After the completion of PCR reaction, five micro liters of stop dye (80% formamide, 50 mM Tris-Hcl (pH=8.8) 1 mM EDTA, 0.1% bromophenol blue and xylene cyanol) was added and the samples were stored at 4 0 C till further use.

Molecular sizes of various alleles of Microsatellite markers were estimated by using 10 bp DNA ladder (Bangalore Genei) / 20 bp DNA ladder (Bangalore Genei)/ 25 bp DNA ladder (Invitrogen) as molecular size marker.

Resolution and documentation of Microsatellite alleles

The amplification products from the microsatellite markers were resolved on 3.5 % metaphor agarose gel. The gels were stained with ethidium bromide and photographed under UV light.

C. Statistical Analysis

Profiling of microsatellite alleles

The alleles at different microsatellite locus were sized using computer software. The allele frequency was estimated as its proportion to the total no of loci (2n), where n is the number of individuals genotyped at that microsatellite loci.

Observed and expected heterozygocity

Observed heterozygocity at a microsatellite locus was measured as proportion of the heterozygous individuals at that microsatellite locus as follows

Observed heterozygocity (Ho) = H / T

Where H is the number of heterozygotes individuals at a locus and T is the total number of individuals genotyped at that locus.

Expected heterozygocity (Ht) at a locus was estimated using an unbiased estimator

$$Hti = (2N/(2N-1) \{ 1 - \sum_{j=1}^{l} P_j 2\}$$

Where P_j is the frequency of j^{th} allele at i^{th} locus with l alleles in population, and N is the number of individuals genotyped at ith locus.

Average hetrozygocity at all loci was calculated from the equation by Nei (1978).

$$H = 1/N \{\sum_{j=1}^{l} Ht_i\}$$

Where Ht_i is the expected heterozygocity at i^{th} locus and N is the total number of loci.

Polymorphic Information Content (PIC)

The PIC was also calculated using microsatellite allelic frequencies as follows

$$PIC = 1 - \sum_{i=1}^{j} P_i 2 - 2 \sum_{i=j+1}^{j} \sum_{j=1}^{i-1} P_i 2 \quad P_j 2$$

Where, P_i and P_j are the frequencies of a i^{th} and j^{th} alleles at a locus in a population, respectively (Botstein et al., 1980).

Estimation of within and between population genetic similarity and genetic distances

The microsatellite marker profile or allelic pattern from the primers that generated polymorphism were utilized. Such allelic patterns were scored for the presence or absence of allele and the data were entered into a binary character matrix. If the allele was present in an individual, it was designated as '1' otherwise as '0'.

The within population genetic similarity (WF) in a population was estimated as band frequency.

$$WF = 1/N \sum_{i=1}^{N} V_i$$

Where, V_i is the frequency of occurrence on i^{th} band and N is the total number of bands scored.

The genetic similarity (WS) between two populations was obtained as follows

$$WS = 1/N \sum_{i=1}^{N} (2.V_i^{(1)}.V_i^{(2)})/[\{V_i^{(1)}\}^2 + \{V_i^{(2)}\}^2]$$

Where $V_i^{(1)}$ and $V_i^{(2)}$ are the frequency of occurrence of i^{th} band in two population 1 and 2, and N is total number of bands scored. The genetic distance, D was estimated as – ln (WS).

IIA. mt DNA polymorphism within as well as between Gallus species

Mt DNA sources and sequence analysis

Complete mitochondrial DNA sequences from different chicken breeds, G. gallus subspecies and Gallus species (Table 2) were retrieved from database (http://www.ncbi.nlm.nih.gov.in). Genes identified for study were 12S ribosomal RNA (12S-rRNA), 16S ribosomal RNA (16S-rRNA), ATPase unit 6 and 8 (ATPase 6 and ATPase 8, respectively), Cytochrome Oxidase subunit I, II and III (COI, COII & COIII, respectively), Cytochrome B (Cytb), NADP dehrdrogenase subunit 1, 2, 3, 4, 4L, 5 and 6 (ND1, ND2, ND3, ND4, ND4L, ND5 and ND6, respectively). Complete mitochondrial sequences were inspected manually and edited using sequence editor option of GENETOOL and the sequences corresponding to the different genes were isolated and saved. Subsequently, the sequences were aligned using CLUSTALW (Thompson et al., 1994), website (http://www.cbi.ac.uk/clustalw/). Jukes-Cantor genetic distances were estimated using the computer program Molecular Evolutionary Genetic Analysis (MEGA Version 2.1). Jukes-Cantor estimates were used because all mitochondrial sequences were not much divergent (< 6 % divergence from raw counts) and no strong transition bias was evident. Phylogenetic trees were constructed with neighbour joining (NJ) procedure using MEGA Version 2.1. Support of the clusters was evaluated by bootstrap, as percentage recurrence of clusters based on 100 bootstrapped replications with MEGA Version 2.1.

IIB. mt DNA polymorphism between Gallus gallus murghi, other Gallus gallus subspecies and Gallus species

Amplification of partial 12S and 16S rRNA genes

Universal primers for mt 16S rRNA gene (Forward-5'- CGC CTG TTT ACC AAA ACA T-3', Reverse-5'- CCG GTC TGA ACT CAG ATC ACG T-3') and for mt 12S rRNA gene (Forward-5'-CAA ACT GGG ATT AGA TAC CCC ACT AT-3', Reverse-5'-GAG GGT GAC GGG CGG TGT GT-3'), were used for PCR amplification. PCR reactions were set up in 25 µl reaction volume containing 2.5 µl of 10 X Assay buffer (100 mM Tris- HC1, pH 9.0, 15 mM MgC12, 500mM KC1 and 0.1% gelatin), 0.20 mM of dNTP mix, 10 pm of forward and reverse primer, 1U Taq DNA polymerase, 50 ng of purified DNA and autoclaved milliQ water to make up the volume. The cycling conditions contained were as follows: initial denaturation at 94 °C for 5 min followed by 30 cycles of 45 sec denaturation at 94 °C, 45 sec annealing at 60 °C and 1 min elongation at 72°C. Amplified products were stored at 4°C till further use.

Resolution of PCR amplified products and documentation

The PCR products were tested for amplification of specific gene by agarose gel electrophoresis using 1.6% agarose gel in 1x TBE. A total volume of 20 ml of 1.6% agarose (Life Technologies Inc.) was prepared in 1x TBE and placed in microwave oven until melted. Molten agarose was allowed to cool to about 45°C and ethidium bromide was added to give a final concentration of 0.5µg/ml. The gel was poured on to electrophoresis trough fitted with comb. The gel was allowed to set on a flat surface for about 15 minutes. Electrophoresis trough was placed in an electrophoresis tank filled with 1x TBE. Samples were prepared on a parafilm by mixing 2 µl loading dye (0.25% w/v bromophenol blue, 0.25% w/v xylene cynol and 40 w/v sucrose in water) and 5 µl of PCR products were loaded in parallel with 100 bp ladder (Gene ruler 100 bp ladder, MBI Fermentas). Electrophoresis was done at 90 volts for 10 minutes, then at 50 volts for 2 hour. Gel was viewed under a UV

Trans-illuminator and photographed with gel documentation system (Syngene) for future analysis.

Gel purification of PCR products

The PCR products were purified from gel using QIAquick Gel Extraction Kit (QIAGEN Inc. Valencia, CA, USA) as follows:

- A preparative gel was prepared in TAE buffer and electrophoresis was done at 30 V for 6 hours. The gel was briefly visualized in low range UV light and the desired band was cut using a sterile paragon blade. The gel slice was collected in a pre-weighed sterile 1.5 ml micro centrifuge tube.
- The gel slice was weighed and 3 volumes of buffer QG was added to 1 volume of gel slice and incubated at 50°C for 10 min. with intermittent vortexing every 2-3 min.
- After the gel slice was completely dissolved, isopropanol equivalent to gel volume was added and mixed.
- Then, the sample was applied to the QIAquick spin column, placed in 2 ml collection tube provided in the kit.
- QIAquick spin column was centrifuged for 1 min at 12000 rpm.
- The flow-through was discarded and the QIAquick spin column was placed back in the same collection tube.
- 500 µl of buffer QG was added to QIAquick spin column and centrifuged for another 1 min at 12000 rpm.
- For washing, 750 µl of Buffer PE was added to column and then centrifuged at 12000 rpm for 1min.
- The flow-through was discarded and the column was placed back in the same collection tube and centrifuged at 12000 rpm for additional 2 min.
- The column was then transferred to a clean 1.5 ml micro centrifuge tube.
- For elution of the Plasmid DNA, 30 µl of buffer EB was added to the center of the column, kept as such for 5 min and then centrifuged at 12000 rpm for 1 min.
- 5 µl of the purified PCR product was run in 1.6% agarose gel in 1x TBE along with 100 bp ladder (GeneRuler 100 bp Ladder, MBI Fermentas).

Cloning of the purified PCR products in pGMT vector

Preparation of ligation reaction

The gel purified PCR products were cloned into pGMT vector system (Promega), as per the manufacturer's protocol. The ligation reaction was prepared in 10 µl reaction in a 0.5ml microcentrifuge tube:

5X Rapid ligation buffer		2.0 µl
pGMT Vector (50 ng)		1.0 µl
Purified PCR product (100ng)		5.0 µl
T4 DNA ligase (5 U)	1.0 µl	
Nuclease free water		2.0 µl

The reaction mixture was incubated at 4°C overnight. The ligated DNA was diluted to 200 µl in 1.1x TCM solutions and kept at 4°C until used for transformation.

Preparation of competent cells and transformation

In order to transform DH5α cells (E. coli cells) with the ligated DNA, fresh competent cells were prepared. The following steps were followed:

- Fresh cultures of DH5α cells were grown in 25ml LB medium in shaking incubator at 37°C overnight.
- The cells were diluted 200 times in LB medium and incubated in shaking incubator at 37°C for about 2-3 hrs until culture Attained an O.D. of 0.3-0.4 at A600 nm.
- The cells were kept on ice for 20 min.
- The cells were pelleted at 6000 rpm for 10 min at 4°C and the pellet was re-suspended in 1/10 volume of chilled TSS solution and incubated on ice for 1 hour.
- Competent cells were then ready for transformation and kept at –20°C until further use.
- 200 µl of competent cells were gently added to the 200µl ligated DNA (diluted in TCM), mixed and incubated on ice for 20 min.
- The DNA-competent cells mixture was subjected to heat shock at 42°C for 45-50 sec. and immediately snap-chilled on ice for 2 min.
- 950 µl SOC medium was added to the transformants and incubated at 37°C for 1.5 hrs with shaking.
- Transformants were then plated on LB/Amp/X-gal/IPTG plates and incubated overnight at 37°C.
- The white colonies were picked on the next day and grown in 10 ml LB/Amp broth for overnight at 37°C with shaking at 150 rpm for plasmid extraction.

Isolation of plasmid DNA

From the over night grown cultures the plasmid DNA was extracted following the small scale alkali lysis method as described by Sambrook et al. (1989) as follows:

- Bacterial culture (1.5 ml twice in the same tube) was centrifuged at 13000 rpm (12000 g) at 4°C for 1 min and supernatant was discarded thoroughly.
- The pellet was re-suspended in 100 µl of ice-cold Solution I by vigorous vortexing. Then 200 µl of freshly prepared Solution II (RT) was added to the suspension and mixed gently by inversion. Next, 150 µl of ice-cold Solution III was added and inverted gently for mixing. The viscous lysate was kept on ice for 3-5 minute.
- The bacterial lysate was centrifuged at 13000 rpm (12000 g) at 4°C for 5 min. The supernatant was transferred to a new microcentrifuge tube.
- Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and mixed well by vortexing. The emulsion was centrifuged at 13000 rpm at 4°C for 2 min and the upper aqueous layer was transferred to a new microfuge tube.
- Equal volume of chloroform: isoamyl alcohol (24:1) was added and centrifuged at 13000 rpm at 4°C for 2 min and the upper aqueous layer was transferred to a new microfuge tube.
- The nucleic acid was precipitated by adding two volumes of ethanol at RT and incubated for 2-5 min at RT after brief vortexing. Then it was centrifuged at 13000 rpm at 4°C for 5 min.
- The supernatant was discarded thoroughly by inverting the tube onto a paper towel and 1 ml of 70% ethanol was added to the pellet. The tube was inverted several times for proper mixing and then centrifuged at 13000 rpm (12000 g) at 4°C for 2 min.
- The supernatant was removed completely and the residual ethanol was evaporated by keeping the tube open at RT for few minutes.
- The plasmid was dissolved in 25 µl TE buffer (pH 8.0) containing 20 µg/ml DNase-free RNase A and briefly vortexed and incubated at RT for 30 min.
- The quality of the plasmid DNA was checked in 1% agarose gel. The plasmid was then stored at -20°C until further use.

Confirmation of cloning by RE digestion

The plasmid DNA, so isolated was subjected to digestion with EcoRI restriction enzyme to recover the inserted fragment. The restriction digestion was done in 0.2 ml thin walled PCR tubes in 10 µl volume. The Procedure was performed as follows:

- To 5 µl of PCR product, 1 µl of restriction enzyme and 2.5 µl of buffer was added. The volume was made 10 µl by adding 1.5 µl ADW to the above mixture.
- Volume was made to 20 µl with autoclaved Milli Q water.
- Reaction mixture was incubated at 37 °C overnight.
- The enzyme activity was stopped by adding 2 µl of 6X loading dye.
- Digested product was subjected to electrophoresis in 1.6% Agarose gel along with 100bp ladder (Bangalore Genei).
- Agarose gel after electrophoresis was observed in gel documentation system for products of desired molecular weight.

Sequencing of amplicon

PCR products from all the five species were sequenced using ABI Prism 377 DNA sequencer at DNA sequencing facility at Bangalore Genei. The nucleotide sequences of 12S rRNA and 16S rRNA gene of different meat species submitted to EMBL (European Molecular Biology Laboratory).

Sequence homology comparisons

The sequences of 12S rRNA and 16S rRNA genes from different domestic fowl breeds, other G. galus subspecies and Gallus species (Table 2) were retrieved from from the database (http://www.ncbi.nlm.nih.gov.in). The 12S rRNA sequence (Accession Number DQ885561) and 16S-rRNA sequence (Accession number DQ867016) were aligned with the respective retrieved sequences using CLUSTALW (Thompson et al., 1994). The unaligned portions towards both the ends were trimmed using DAMBE (Xia, X., and Xie. Z., 2001) . Nucleotide diversity between the sequences was measured in terms of Jukes-Cantor distance using Molecular Evolutionary Genetic Analysis (MEGA Version 2.1). Phylogenetic trees were constructed with neighbour joining (NJ) procedure using MEGA Version 2.1 as described above. The sequence of 12S rRNA and 16S rRNA from guinea fowl (Numidae meleagris) were used as out-group for developing the phylogenetic tree.

Table 1. Details of the microsatellite locus used in present study

Locus	Chromosome	Position (cM)	Sequence (5'-3')
ADL268	1	288	CTC CAC CCC TCT CAG AAC TA
			CAA CTT CCC ATC TAC CTA CT
MCW111	1	118	GCT CCA TGT GAA GTG GTT TA
			ATG TCC ACT TGT CAA TGA TG
MCW248	1	20	GTT GTT CAA AAG AAG ATG CAT G
			TTG CAT TAA CTG GGC ACT TTC
MCW34	2	230	TGC ACG CAC TTA CAT ACT TAG AGA
			TGT CCT TCC AAT TAC ATT CAT GGG
MCW206	2	104	CTT GAC AGT GAT GCA TTA AAT
			ACA TCT AGA ATT GAC TGT TC
MCW37	3	317	ACC GGT GCC ATC AAT TAC CTA TTA
			GAA AGC TCA CAT GAC ACT GCG AAA
MCW103	3	210	AAC TGC GTT GAG AGT GAA TGC
			TTT CCT AAC TGG ATG CTT CTG
MCW222	3	86	GCA GTT ACA TTG AAA TGA TTC C
			TTC TCA AAA CAC CTA GAA GAC
MCW98	4	217	GGC TGC TTT GTG CTC TTC TCG
			CGA TGG TCG TAA TTC TCA CGT
MCW284	4	167	CAG AGC TGG ATT GGT GTC AAG
			GCC TTA GGA AAA ACT CCT AAG G
MCW295	4	75	ATC ACT ACA GAA CAC CCT CTC
			TAT GTA TGC ACG CAG ATA TCC
MCW81	5	123	GTT GCT GAG AGC CTG GTG CAG
			CCT GTA TGT GGA ATT ACT TCT C
LEI192	6	114	TGC CAG AGC TTC AGT CTG T
			GTC ATT ACT GTT ATG TTT ATT GC
MCW14	6	96	AAA ATA TTG GCT CTA GGA ACT GTC
			ACC GGA AAT GAA GGT AAG ACT AGC
MCW183	7	79	ATC CCA GTG TCG AGT ATC CGA
			TGA GAT TTA CTG GAG CCT GCC
ADL278	8	87	CCA GCA GTC TAC CTT CCT AT
			TGT CAT CCA AGA ACA GTG TG
MCW78	8	87	CCA CAC GGA GAG GAG AAG GTC T
			TAG CAT ATG AGT GTA CTG AGC TTC
ADL112	10	0	GGC TTA AGC TGA CCC ATT AT
			ATC TCA AAT GTA ATG CGT GC
MCW67	10	61	GCA CTA CTG TGT GCT GCA GTT T
			GAG ATG TAG TTG CCA CAT TCC GAC
MCW216	13	28	GGG TTT TAC AGG ATG GGA CG
			AGT TTC ACT CCC AGG GCT CG
MCW330	17	41	TGG ACC TCA TCA GTC TGA CAG
			AAT GTT CTC ATA GAG TTC CTG C
MCW69	E60C04W23	23	GCA CTC GAG AAA ACT TCC TGC G
			ATT GCT TCA GCA AGC ATG GGA GGA

Table 2. Details of accession numbers used

Species	**Subspecies**	**Breeds**	**Common name**	**Accession number**
Gallus varius	-	-	Green jungle fowl	AP003324
Gallus lafayettei	-	-	Ceylon jungle fowl	AP003325
Gallus sonneratii	-	-	Grey jungle fowl	AP006741
Gallus gallus	Gallus gallus gallus	-	Cochin Chinese red jungle fowl	AP003322
	Gallus gallus gallus	-	Cochin Chinese red jungle fowl	NC_007236
	Gallus gallus bankiva	-	Javan red jungle fowl	AP003323
	Gallus gallus spadicus	-	Burmese Red jungle fowl	AP003321
	Gallus gallus domesticus	White Leghorn (WLH)	Domestic fowl	AP003317
	Gallus gallus domesticus	White Plymouth Rock (WPR)	Domestic fowl	AP003318
	Gallus gallus domesticus	Native Laos (NL)	Domestic fowl	AP00319
	Gallus gallus domesticus	White Leghorn (WLH)	Domestic fowl	AP003580
	Gallus gallus domesticus	New Hampshire (NH)	Domestic fowl	AY235570
	Gallus gallus domesticus	White Leghorn (WLH)	Domestic fowl	X52392
	Gallus gallus domesticus	Tibetan (T)	Domestic fowl	DQ648776
	Gallus gallus domesticus	Silky (SL)	Domestic fowl	AB86102

Results

Results

I. Microsatellite polymorphism between red jungle fowl and domestic chicken breeds

A. Microsatellite Polymorphism

A total of 22 dinucleotide (CA)n repeat microsatellite markers were used to genotype the resource population. The total number of loci, allelic size range, polymorphic information content (PIC), observed and expected heterozygocity in red jungle fowl, other chicken breeds and across the whole populations at different microsatellite locus have been presented in Table 3 to 24. Out of these markers, 21 markers were found to be polymorphic across the whole population, while MCW 103 showed no polymorphism. The

microsatellite allelic profiles in different population at these loci are shown in Fig 1 to 22.

Microsatellite ADL 268

At ADL 268 locus, 5-7 alleles were observed in different populations, while across the population, total numbers of alleles were 10. The size range across the populations was 100-120 bp. PIC value was moderate to high and ranged from 0.472 to 0.791 in different populations. Observed heterozygocity ranged from 0.200 to 0.866, while the expected hetrozygocity ranged from 0.484 to 0.804 in different populations. In RJF, low heterozygocity was observed at this locus in comparison to domestic chicken breeds. These estimates were 0.862, 0.427 and 0.869, respectively across the populations (Table 3).

Microsatellite MCW 111

For MCW 111 marker, 4-5 alleles were found in different populations, while across the population, total numbers of alleles were 6 in the range of 98 bp to 108 bp. PIC value was moderately high and ranged from 0.385 to 0.711 in different populations. While the observed heterozygocity ranged from 0.231 to 0.636, the expected hetrozygocity was 0.393 to 0.733 in different populations. In RJF and KN, comparatively higher heterozygocity was observed in comparison to other breeds. These estimates were 0.725, 0.433 and 0.743, respectively across the populations (Table 4).

Microsatellite MCW 248

MCW 248 microsatellite marker showed only 2 alleles in WL, while in other populations, 5-6 alleles were observed. Across the population total alleles were 12. The size ranges of these alleles were from 210 bp to 238 bp. PIC values was low to high and ranged from 0.128 to 0.752 in different populations. While the observed heterozygocity ranged from 0.000 to 0.666, the expected hetrozygocity was 0.133 to 0.771 in different populations. In WL, very low heterozygocity was observed in comparison to other breeds

including RJF. These estimates were 0.876, 0.397 and 0.881, respectively across the populations (Table 5).

Microsatellite MCW 34

or MCW 34, the number of alleles ranged from 6 in WL to 14 in KN, however across the populations total number of alleles were 18. The size range across the population was 230 bp to 272 bp. PIC values were high and ranged from 0.716 to 0.899 in different populations. Observed heterozygocity (0.231 to 0.636) as well as expected hetrozygocity (0.816-0.904) was high and more or less similar in all the populations. These estimates were 0.897, 0.907 and 0.922, respectively across the populations (Table 6).

Microsatellite MCW 37

For MCW 37 marker, 4-8 alleles were found in different populations, while across the population, total numbers of alleles were 13 and the allelic size range was from 144 bp to 178 bp. PIC values were high and ranged from 0.679 to 0.838 in different populations. While the observed heterozygocity was comparatively low (0.000 to 0.333), the expected hetrozygocity was high (0.704-0.847) in different populations. In all the populations, more or less heterozygocity of similar magnitude was observed. These estimates were 0.907, 0.164 and 0.910, respectively across the populations (Table 7).

Microsatellite MCW 206

At MCW 206 locus, 3 to 7 alleles were found in different populations, while across the population total number of alleles were 10 with the allelic size range between 206 bp to 242 bp. PIC values were moderately high and ranged from 0.418 to 0.810 in different populations. While the observed heterozygocity (0.071 to 0.400) was low, expected hetrozygocity was comparatively high (0.457 to 0.822) in different populations. In RJF, low heterozygocity was observed in comparison to other chicken breeds. These estimates were 0.837, 0.200 and 0.845, respectively across the populations (Table 8).

Microsatellite MCW 222

For MCW 222 marker, comparatively fewer alleles were found. While only one allele was present in RC, 2-5 alleles were found in other populations. Across the populations, total number of alleles was 5 and the allelic size range was 220 bp - 254 bp. PIC values were moderate and ranged from 0.353 to 0.653 in different populations. While the observed heterozygocity ranged from 0.000 to 0.733, the expected hetrozygocity was 0.000 to 0.589 in different populations. In RC, no heterozygocity was observed, while in other breeds including RJF, more or less similar heterozygocity was observed. These estimates were 0.803, 0.310 and 0.591, respectively across the populations (Table 9).

Microsatellite MCW 98

For MCW 98 marker, 2-5 alleles were found in different populations, while across the population, total numbers of alleles were 6 and the allelic size range was from 242 bp to 274 bp. PIC values were low to moderate and ranged from 0.353 to 0.653 in different populations. While the observed heterozygocity was zero, the expected hetrozygocity was moderate (0.391 to 0.673) in different populations and was more or less similar in different populations. These estimates were 0.803, 0.000 and 0.816, respectively across the populations (Table 10).

Microsatellite MCW 284

Similarly, at MCW 284 locus, though the numbers of alleles in different populations were few (3-5), but across the populations, total number of alleles was comparatively more i.e. 11. The size range across the population was 372 bp to 428 bp. PIC values was low to moderately high and ranged from 0.259 to 0.754 in different populations. Observed heterozygocity was low and ranged from 0.000 to 0.400, the expected hetrozygocity was low to moderately high (0.269 to 0.748) in different populations. In AS, low heterozygocity was observed in comparison to other breeds including RJF.

These estimates were 0.872, 0.191 and 0.878, respectively across the populations (Table 11).

Microsatellite MCW 295

For MCW 295, the number of alleles ranged from 2 in RC to 8 in WL, however across the populations total number of alleles were 12. The size range across the population was 140 bp to 164 bp. PIC values was low to high and ranged from 0.172 to 0.790 in different populations. Observed heterozygocity (0.200 to 0.667) as well as expected hetrozygocity (0.180 to 0.804) was low to moderately high in different populations. In RC, low heterozygocity was observed in comparison to other breeds including RJF. These estimates were 0.841, 0.427 and 0.849, respectively across the populations (Table 12).

Microsatellite MCW 81

For MCW 81 marker, 2-6 alleles were found in different populations, while across the population, total numbers of alleles were 7 and the allelic size range was from 110 bp to 146 bp. PIC values was low to high and ranged from 0.121 to 0.702 in different populations. While the observed heterozygocity ranged from 0.133 to 0.933, the expected hetrozygocity was 0.124 to 0.724 in different populations. In RC and AS, low heterozygocity was observed in comparison to other breeds including RJF. These estimates were 0.732, 0.480 and 0.752, respectively across the populations (Table 13).

Microsatellite LEI 192

At LEI 192, 21 alleles were present in across the population, while number of alleles in different populations were comparatively much lower i.e. 6-8. The allelic size pattern differed between the populations. While in RJF, the allelic size was from 352 – 468 bp, other populations showed alleles in the size range of 218 bp to 358 bp. PIC values was moderately high and ranged from 0.570 to 0.797 in different populations. While the observed heterozygocity ranged from 0.267 to 0.733, the expected hetrozygocity was 0.727 to 0.840 in different populations. In all the populations, more or less similar heterozygocity

was observed. These estimates were 0.902, 0.480 and 0.928, respectively across the populations (Table 14).

Microsatellite MCW 14

For MCW 14, the number of alleles ranged from 2 in WL to 8 in KN and RJF, however across the populations total number of alleles were 15. The size range across the population was 168 bp to 206 bp. PIC values was moderate to high and ranged from 0.353 to 0.840 in different populations. While the observed heterozygocity ranged from 0.000 to 0.667, the expected hetrozygocity was 0.391 to 0.849 in different populations. In WL, comparatively low heterozygocity was observed in comparison to other breeds including RJF. These estimates were 0.872, 0.405 and 0.878, respectively across the populations (Table 15).

Microsatellite MCW 183

For MCW 183 marker, 4-14 alleles were found in different populations, while across the population, total number of alleles was 19 and the allelic size range was from 268 bp to 328 bp. PIC values was high and ranged from 0.528 to 0.882 in different populations. While the observed heterozygocity ranged from 0.133 to 0.733, the expected hetrozygocity was 0.551 to 0.889 in different populations. In different populations, more or less similar heterozygocity was observed. These estimates were 0.886, 0.419 and 0.901, respectively across the populations (Table 16).

Microsatellite MCW 78

For MCW 78 marker, 2-6 alleles were found in different populations, while across the population, total number of alleles was 6 and the allelic size range was from 140 bp to 152 bp. PIC values was low to high and ranged from 0.121 to 0.739 in different populations. While the observed heterozygocity ranged from 0.133 to 0.466, the expected hetrozygocity was 0.124 to 0.758 in different populations. In AS, low heterozygocity was observed in comparison to other breeds including RJF. These estimates were 0.789, 0.200 and 0.801, respectively across the populations (Table 17).

Microsatellite ADL 278

At ADL 278 locus, number of alleles were 3 to 6 in different population and across the population, a total of 7 alleles were present. Acroos the population, allelic size range was 114 bp to 136 bp. PIC values was moderately high and ranged from 0.313 to 0.791 in different populations. While the observed heterozygocity ranged from 0.133 to 0.429, the expected hetrozygocity was 0.331 to 0.804 in different populations. These estimates were 0.738, 0.239 and 0.753, respectively across the populations (Table 18).

Microsatellite ADL 112

For ADL 112 marker, 2-5 alleles were found in different populations, while across the population, total number of alleles was 7 and the allelic size range was from 122 bp to 134 bp. PIC values was moderate to high and ranged from 0.357 to 0.718 in different populations. While the observed heterozygocity was very low (0.000 to 0.133), the expected hetrozygocity was moderate to high (0.397 to 0.735) in different populations. In different populations, more or less similar heterozygocity was observed in comparison to other breeds including RJF. These estimates were 0.770, 0.030 and 0.785, respectively across the populations (Table 19).

Microsatellite MCW 67

At MCW 67 locus, though the numbers of alleles in different populations were more or less similar (6-8), but across the populations, total number of alleles was comparatively more i.e. 14. The size range across the population was 170 bp to 200 bp. PIC values were high and ranged from 0.685 to 0.810 in different populations. While the observed heterozygocity ranged from 0.267 to 0.733, the expected hetrozygocity was 0.704 to 0.764 in different populations. In different populations, more or less similar hetrozygocity was observed. These estimates were 0.903, 0.472 and 0.906, respectively across the populations (Table 20).

Microsatellite MCW 216

For MCW 216 marker, comparatively fewer alleles were found. While only one allele was present in WL, 2-4 alleles were found in other populations. Across the populations, total number of alleles was 6 and the allelic size range was 142 bp - 144 bp. PIC values were moderately high (0.368 to 0.645) in most of the populations. While the observed heterozygocity was low (0.133 to 0.267), the expected hetrozygocity moderate (0.384 to 0.678) in different populations, except WL, in which observed as well as expected heterozygocity were zero. These estimates were 0.747, 0.147 and 0.763, respectively across the populations (Table 21).

Microsatellite MCW 330

At MCW 330 locus, though the number of alleles ranged only between 6 to 9 in different populations, total number of alleles was more i.e. 17 across the populations. . The size range across the population was 140 bp to 164 bp. PIC values was high and ranged from 0.737 to 0.832 in different populations. Observed heterozygocity as well as expected heterozygocity was high and ranged from 0.333 to 0.533 and 0.764 to 0.852 in different populations. In different populations more or less similar heterozygocity was observed. These estimates were 0.906, 0.432 and 0.912, respectively across the populations (Table 22).

Microsatellite MCW 69

For MCW 69 marker, 6-9 alleles were found in different populations, while across the population, total number of alleles was 11 and the allelic ranged from 154 bp to 180 bp in size. PIC values were high and ranged from 0.600 to 0.804 in different populations. Observed heterozygocity as well as expected heterozygocity was high and ranged from 0.333 to 0.636 and 0.611 to 0.816 in different populations. In different populations more or less similar heterozygocity was observed. These estimates were 0.836, 0.507 and 0.844, respectively across the populations (Table 23).

B. Population specific alleles

Allelic frequencies of different alleles at various microsatellite loci in different populations are presented in Table 25 to 45. At ADL 268 locus, out of the 10 alleles, no allele was specific to any population. Out of the 6 alleles present at MCW 111 locus, none of the allele was specific to any population. At MCW 248 locus, three population specific alleles were observed. While 210 bp allele was specific to KN, 222 bp, 230 bp and 238 bp alleles were specific to AS. Three RJF specific alleles i.e. 230 bp, 236 bp and 246 bp were identified at MCW 34 locus. The allelic frequencies of these alleles were 0.23, 0.06 and 0.23, respectively. At MCW 37 locus, only one WL specific allele (176 bp) was identified. At MCW 206, one AS specific allele (208 bp) with a frequency of 0.23 was identified. Further the alleles of lower size i.e. 206 bp to 218 bp were absent in RJF and present in different chicken breeds. At MCW 222 locus, only one KN specific allele (226 bp) was identified, while no population specific allele could be identified at MVW 98 locus. At MCW 284 locus, three population specific alleles were identified. While the two alleles i.e. 372 bp and 428 bp were specific to RJF, 394 bp allele was specific to RC. AT MCW 295 locus, 5 population specific alleles were observed. The 144 bp allele was RJF specific and present in high frequency (0.50). Among other population specific alleles, 2 were specific to WL i.e. 140 bp (0.03) and 154 bp (0.37); one was specific to KN i.e. 152 bp (0.13) and one was specific to AS i.e. 152 bp (0.10). At MCW 81 no population specific allele was observed. At LEI 192 locus, 10 population specific alleles were identified and 6 of which were specific to RJF i.e. 368 bp, 400 bp, 426 bp, 444 bp, 452 bp and 468 bp. The cumulative allelic frequency of these alleles was 0.88. Among other population specific alleles, 3 were specific to WL i.e. 218 bp, 232 bp and 272 bp and one was specific to AS i.e. 304 with a frequency of o,37. Lower size alleles i.e. 218 bp to 326 bp were completely absent in RJF, but present in other chicken breeds at varying frequencies. Similarly, at MCW 14 locus, 7 population specific alleles were found and 4 of these were RJF specific. The RJF specific alleles i.e. 196 bp, 202 bp, 204 bp and 206 bp were present at

0.40 frequency. Among other population specific alleles, 2 were KN specific i.e. 168 bp and 180 bp, while one if RC specific i.e. 174 bp. Out of the 18 alleles at MCW 183 locus, one allele i.e. 274 bp was specific to RJF and had the frequency of 0.14. Other 3 alleles i.e. 318 b0, 320 bp and 322 bp were specific to AS and had a cumulative frequency of 0.23. No population specific allele could be observed at MCW 78 and MCW 278 loci. Out of 7 alleles at ADL 112, one was specific to RJF with a frequency of 0.27, while other was specific to AS (0.07). Out of the 3 population specific alleles at MCW 67 locus, one was specific to RJF i.e. 184 bp with the frequency of 0.4 while the other allele i.e. 194 bp was specific to WL, though had a very low frequency of 0.035. At MCW 216 locus, 1 RJF specific allele i.e. 158 bp was identified. Out of 17 loci at MCW 330, 4 were population specific. While 250 bp allele was RJF specific and had a frequency of 0.25, 234 bp and 242 bp alleles were WL specific and had the frequencies og 0.03 and 0.17, respectively. The 266 bp allele was specific to RC and had the frequency of 0.33. At MCW 67 locus, no population specific allele cold be observed, though the three low size alleles i.e. 154 bp, 156 bp and 158 bp were completely absent in RJF and present in other chicken breeds, but at low frequency.

C. Genetic Relatedness between the RJF and other chicken breeds

Based on the microsatellite profile, a matrix of "0" and "1" was made for each markers. While the "0" showed the absence of a band, "1" indicated the presence of a band in an individual. The within as well as between population genetic similarity was estimated on the basis of the band frequency.

Within population genetic similarity

The estimates of within population genetic similarity (WF) in different populations microsatellite markers wise as well as pooled over the markers have been presented in Table 46 to 67. The estimates differed between the

markers and between the populations within the marker. On the basis of band frequencies, at most of the microsatellite loci, the within population genetic similarity estimates were low to moderate i.e. between 0.200 – 0.400. At some of the microsatellite loci i.e. MCW 69, MCW 330 very low within population genetic similarity (> 0.200) were estimated, however some locus such as MCW 222 and MCW 216, moderate to high within population genetic similarity estimates (0.50 to 1.00) were estimated. Within population genetic similarity estimates, pooled over the markers were 0.289, 0.314, 0.217, 0.324 and 0.360 in RJF, WL, KN, AS and RC populations, respectively.

Between population genetic similarity and genetic distance

The estimates of genetic similarity between different populations (BF) with different microsatellite markers as well as pooled over the markers have been presented in Table 46 to 67. Estimates differed between the markers and between the populations within the marker. In general low to moderate estimates of between population genetic similarity were observed. Pooled over the markers, the BF estimates ranged from 0.181 to 0.312. RJF showed low genetic similarity with the chicken breeds. At this low level of genetic similarity, WL seemed to be more close to RJF and RC seemed to most distant. Among the different chicken breeds, WL showed very low genetic similarity with AS and RC (0.182 – 0.185) in comparison to KN (0.312). Similarly RC showed more genetic similarity with KN and AS (0.256 – 0.298) in comparison to RJF and KN (0.181 – 0.185). Both the native breeds showed more genetic similarity with each other (0.303) in comparison to other breeds.

The genetic distances between different populations were estimated as negative log of the genetic similarity estimates and are presented in Table 46 to 67. Estimates differed between the markers and between the populations within the marker. In general very high estimates of between population genetic distances were observed. Pooled over the markers, the estimates ranged from 1.193 to 1.705 and reflected more or less similar trend.

IIA.mt DNA polymorphism within as well as between *Gallus* species

The nucleotide sequences of different mitochondrial genes were aligned from different chicken breeds, *gallus gallus* sub species and *gallus* species (Table 2) were aligned and compared. The nucleotide sequence variation among them for different mitochondria genes has been presented in Table 68.

A. Nucleotide diversity between chicken breeds (*G. g. domesticus*)

12S- rRNA gene

The 12SrRNA showed no size variation in different domestic chicken breeds (*G. g. domesticus*). Between different chicken breeds, over 976 nucleotides, only three nucleotide substitutions i.e. G by A in New Hampshire, G by A in White Leghorn(1) and C by T in Silky as well as in White Leghorn (3) were found. All these substitutions were transitions.

16S- rRNA gene

For 16S rRNA, size variation was observed within domestic chicken breeds and the size ranged from 1621 to 1626 bp. In Silky and Native Laos chicken, the 16S rRNA was 1622 bp, while with one deletion it was 1621 bp in Tibetian and New Hampshire breeds. In White Leghorn(3), two deletions and one insertions made 16S rRNA 1621 bp long, while insertions of 1, 3 and 4 nucleotides, resulted in length of 16S rRNA to be 1623, 1625 and 1626 bp in White Plymouth Rock, White Leghorn(2) and White Leghorn(3), respectively. There was only three nucleotide substitutions (all transitions) which were confined to only White Leghorn(3) and Silky breeds over a length of 1621-1626 nucleotides.

ATPase genes

For ATPase6 and ATPase8, no size variations was observed between the different chicken breeds and the size was 684 bp ad 165 bp respectively.

Very low amount of nucleotide substitution was observed between domestic chicken breeds for ATPase6. Three nucleotide substitutions (A by G) were observed in Silky and White Leghorn(3), while other breeds showed no nucleotide sequence variation. The ATPase8 was found to be completely conserved in domestic chicken breeds.

Cytochrome Oxidase genes

For all cytochrome oxidase genes, no size variation was observed between chicken breeds, except COIII. For COIII, insertions of 2 and 3 nucleotides at extreme 3' end caused its size to be 786 bp and 787 bp in Native Laos and New Hampshire chicken, respectively. The Size of COI and COII was 1551 bp and 684 bp, respectively in all the chicken breeds.

For all the cytochrome oxidase genes, very low amount of nucleotide variability was observed between different chicken breeds. For COI, over 1551 nucleotides, substitutions were observed at 7 sites only and 6 of them were transitions, while 1 was transversion. Similarly for COII, out of 684 nucleotides, substitutions were observed at 3 sites. While 2 of them were transitions, 1 was transversion. Very low amount of nucleotide variability was observed between them for COIII as over 787 nucleotides, substitutions were found at 3 sites. Two of them (G by A and C by T) were confined to White Leghorn(3) and Silky, while one (G by A) was present in Tibetian chicken. All these nucleotide substitutions were transitions.

Cytochrome B

For CYTB, no size variation was observed between chicken breeds. Very low amount of nucleotide variability was observed between them. Nucleotide substitutions at 2 sites were observed. While the substitution of T by C was observed in Silky and Tibetian chicken, the A is replaced by G in New Hampshire. Both the nucleotide substitutions were transitions.

NADH dehydrogenase genes

No size variation was observed for all the NADH dehrogenase genes except ND4. In ND4, 2 insertions at extreme 3' end in Native Laos chicken increased the size of ND4 gene to 1380 in comparison to 1378 bp in other chicken breeds. For other NADH dehrogenase genes i.e. ND1, ND2, ND3, ND4L, ND5 and ND6, the sizes were 975 bp, 1041 bp, 351 bp, 297 bp, 1818 bp and 522 bp, respectively.

For all the NADH dehrogenase genes, very low amount of nucleotide variability was observed between different chicken breeds. For ND1, only one nucleotide substitution of A by G (transition) was found in Silky and White Leghorn(3) over 975 nucleotides. Similarly for ND2, one nucleotide substitution of A by C (transversion) was found in White Leghorn(3) and one nucleotide substitution of T by G was found in Silky chicken over 1041 nucleotides. Two nucleotide substitutions were observed over 351 nucleotides of ND3 gene. This substitutions were limited to Silky, White Leghorn(3) and Tibetan only and both were transitions. For ND4, substitutions were observed at only 6 sites over 1378-1380 nucleotides and all were transitions, mainly confined to Silky and White Leghorn(3) only. For ND4L, one nucleotide substitution of T by C (transition) was found in Silky, White Leghorn(3) and Tibetian chicken over 297 nucleotides. For ND5, very low amount of nucleotide variability was observed between them as nucleotide substitutions were observed at 6 sites only. Out of these 6 nucleotide substitutions, 5 were transitions and 1 was transversion. Similarly, very low amount of nucleotide variability was observed for ND6 as nucleotide substitutions were observed at 4 sites only. All the nucleotides substitutions were transitions and lomited to Silky, Tibetian and White Leghorn(3).

B. Nucleotide diversity between different *G. gallus* subspecies

12S- rRNA gene

The 12SrRNA showed no size variation in different *G. gallus* subspecies. Between three red jungle fowl subspecies, one transition (T by C) and one transversion (C by A) were found in *G. g. spadiceus* only, while other two subspecies showed no nucleotide variation between them. It suggested much low nucleotide diversity between other subspecies of *G. gallus*. Jukes-Cantor genetic distances between different *G. gallus* subspecies were quite low and ranged from 0.000 to 0.001,

16S- rRNA gene

Among red jungle fowl subspecies, the size of 16S-rRNA was consistently 1622 bp. Similarly, within red jungle fowl subspecies, only two nucleotide substitutions (A by G) in *G. g. spadiceus* only, while other two red jungle fowl species showed no nucleotide sequence variation. It revealed very high nucleotide similarity among them.

ATPase genes

For ATPase6 and ATPase8, no size variation was observed between the different *G. gallus* subspecies and the size was 684 bp ad 165 bp respectively. Very low amount of nucleotide substitution was observed between these subspecies for ATPase6 as only one transition (A by G) was observed in *G. g. spadiceus*, while two subspecies showed no nucleotide sequence variation. The ATPase8 was found to be completely conserved in all the three *G. gallus* subspecies.

Cytochrome Oxidase genes

No size variation was observed for all the cytochrome oxidase genes. The size of these genes i.e. COI, COII and COIII were 1551 bp, 684 bp and 786 bp, respectively. For COI, extremely low amount of nucleotide variability was observed between subspecies of red jungle fowl, as only one nucleotide

substitution (G by A) was observed in *G. g. bankiva*, while other two subspecies showed no nucleotide sequence variation. For COII, extremely low amount of nucleotide variability was observed between subspecies of red jungle fowl, as only one nucleotide substitution (T by C) was observed in *G. g. spadiceus*, while other two subspecies showed no nucleotide sequence variation. For COIII, very low amount of nucleotide variability was observed between subspecies of red jungle fowl, as only two nucleotide substitutions were observed between them. One substitution (G by A) was in *G. g. bankiva*, while another substitution (G by A) was in *G. g. spadiceus*. Both the nucleotide substitutions were transitions.

Cytochrome B

For CYTB, no size variation was observed between red jungle fowl species. Very low amount of nucleotide variability was observed between them. Nucleotide substitutions at 4 sites were observed. While in *G. g. spadiceus*, 2 nucleotide substitutions (T by A and C by T), *G. g. bankiva* also showed 2 nucleotide substitutions (T by C and C by T). Similarly, *G. g. gallus* showed two nucleotide substitutions I.e. T by C and C by T. Among these nucleotide substitutions, 3 were transitions and 1 was transversion.

NADH dehydrogenase genes

No size variation was observed for all the NADH dehrogenase genes. The size of these NADH dehrogenase genes i.e. ND1, ND2, ND3, ND4, ND4L, ND5 and ND6, the sizes were 975 bp, 1041 bp, 351 bp, 1380 bp, 297 bp, 1818 bp and 522 bp, respectively. For ND1, no size variation was observed between red jungle fowl subspecies. Very low amount of nucleotide variability was observed between them as only two nucleotide substitutions i.e. T by C and A by G was found in *G. g. gallus* and *G. g. bankiva*. Both the nucleotide substitutions were transitions, For ND2, no size variation was observed between different red jungle fowl subspecies. Very low amount of nucleotide variability was observed between them as one nucleotide substitution of T by C (transition) and one of G by T (Transversion) was found in

G. g. spadiceus only. For ND3, no size variation was observed between different red jungle fowl subspecies. Very low amount of nucleotide variability was observed between them as one nucleotide substitution of C by T (transition) was found in *G. g. spadiceus* only.

For ND4, no size variation was observed different red jungle fowl subspecies as the size was 1380 in all the subspecies. Low amount of nucleotide variability was observed between them as at only 3 sites nucleotides substitutions were observed and all were transitions. For ND4L, no size variation was observed different red jungle fowl subspecies. Low amount of nucleotide variability was observed between them as at only 3 sites nucleotides substitutions were observed and 2 of them were transitions, while the one was transversion. For ND5, no size variation was observed between red jungle fowl subspecies. Very low amount of nucleotide variability was observed between them as one nucleotide substitution of T by C (transition) was found in *G. g. bankiva* only. For ND6, no size variation was observed between different red jungle fowl subspecies. Very low amount of nucleotide variability was observed between them as one nucleotide substitution of C by T (transition) was found in *G. g. bankiva* and one of G by A (Transition) was found in *G. g. spadiceus* only.

C. Nucleotide diversity between different Gallus species

12S- ribosomal RNA gene

Size variation was observed in other jungle fowls. With one deletion and three insertions, 12S rRNA was 978 bp in *G. sonneratii*, while with one deletion and two insertions, it was 977 bp in *G. lafayetei*. In *G. varius*, the deletions reduced the size to 973 bp. In *G. lafayetei*, 32 nucleotide substitutions (30 transitions and 2 transversions), in *G. sonneratii*, 33 nucleotides substitutions (all transitions) and in *G. varius*, 31 nucleotide substitutions (30 transitions and 1 transversions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, 12 were fixed in these jungle fowls and all were transitions.

16S- ribosomal RNA gene

In other jungle fowl, size varied from 1618 to 1622. *G. varius* showed two insertions and 2 deletions, while *G. lafayetei*, had 4 deletions and 2 insertions. Similarly, *G. sonneratii* 5 deletions and one insertions. These indels resulted in the length of 16S rRNA to be 1622, 1620 and 1618 in *G. varius*, *G. lafayetei* and *G. sonneratii*, respectively. In *G. lafayetei*, 27 nucleotide substitutions (24 transitions and 3 transversions), in *G. sonneratii*, 57 nucleotides substitutions (54 transitions and 3 transversions) and in *G. varius*, 47 nucleotide substitutions (42 transitions and 5 transversions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, 15 were fixed in these jungle fowls and out of these, 13 were transitions and 2 were transitions.

ATPase genes

For both the genes, no size variations was observed between different jungle fowls for ATPase6 as well as ATPase8 and the respectices sizes were 684 and 165 bp. For ATPase6, in *G. lafayetei*, 20 nucleotide substitutions (19 transitions and 1 transversions), in *G. sonneratii*, 28 nucleotides substitutions (24 transitions and 4 transversions) and in *G. varius*, 23 nucleotide substitutions (21 transitions and 2 transversions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, 6 were fixed in these jungle fowls and out of these, 5 were transitions and 1 was transitions. For ATPase8, a total of 12, 8 and 11 nucleotides substitutions were observed in *G. lafayetei*, *G. sonneratii* and *G. varius*, respectively. All these nucleotide substitutions were transitions. Among these nucleotide substitutions, 7 were fixed in these jungle fowls and out of these, 5 were transitions and 1 was transitions.

Cytochrome oxidase genes

For cytochrome oxidase genes, no size variation was observed between the different jungle fowls, except COIII. For COIII, insertions of 2 nucleotides at extreme 3' end in *G. varius* and *G. lafayetei* resulted in increase of COIII to 786 bp in comparison to 784 bp in *G. sonneratii*. The Size of COI and COII was 1551 bp and 684 bp, respectively in all the jungle fowl

species. For COI, in *G. lafayetei*, 51 nucleotide substitutions (45 transitions and 6 transversions), in *G. sonneratii*, 82 nucleotides substitutions (78 transitions and 4 transversions) and in *G. varius*, 30 nucleotide substitutions (28 transitions and 2 transversions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, 6 were fixed in these jungle fowls and all were transitions. For COII gene, no size variation was observed between the different jungle fowls. For COII, in *G. lafayetei*, 27 nucleotide substitutions (26 transitions and 1 transversions), in *G. sonneratii*, 25 nucleotides substitutions (24 transitions and 1 transversions) and in *G. varius*, 28 nucleotide substitutions (25 transitions and 3 transversions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, 8 were fixed in these jungle fowls and all were transitions. For COIII, in *G. lafayetei*, 43 nucleotide substitutions (36 transitions and 7 transversions), in *G. sonneratii*, 42 nucleotides substitutions (38 transitions and 4 transversions) and in *G. varius*, 47 nucleotide substitutions (42 transitions and 5 transversions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, 17 were fixed in these jungle fowls, out of which 15 were transitions and 2 were transversions.

Cytochrome B

For CYTB, no size variation was observed between different jungle fowl species. In *G. lafayetei*, 59 nucleotide substitutions (51 transitions and 8 transversions), in *G. sonneratii*, 54 nucleotides substitutions (50 transitions and 4 transversions) and in *G. varius*, 43 nucleotide substitutions (36 transitions and 7 transversions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, 16 were fixed in these jungle fowls, out of which 15 were transitions and 1 was transversions.

NADH dehydrogenase genes

No size variation was observed for all the NADH dehrogenase genes except ND4. In ND4, the size variation was due to 2 insertions at extreme 3' end in *G. lafayetei* and *G. varius*, which increased the size of ND4 gene in

these species to 1380. For other NADH dehrogenase genes i.e. ND1, ND2, ND3, ND4L, ND5 and ND6, the sizes were 975 bp, 1041 bp, 351 bp, 297 bp, 1818 bp and 522 bp, respectively.

For ND1, in *G. lafayetei*, 51 nucleotide substitutions (49 transitions and 2 transversions), in *G. sonneratii*, 55 nucleotides substitutions (51 transitions and 4 transversions) and in *G. varius*, 19 nucleotide substitutions (all transitions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, 5 were fixed in these jungle fowls and all of them were transitions. For ND2, in *G. lafayetei*, 55 nucleotide substitutions (49 transitions and 6 transversions), in *G. sonneratii*, 53 nucleotides substitutions (48 transitions and 5 transversions) and in *G. varius*, 23 nucleotide substitutions (19 transitions and 4 transversions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, 11 were fixed in these jungle fowls, out of which 10 were transitions and 1 was transversion. For ND3, in *G. lafayetei*, 16 nucleotide substitutions (all transitions), in *G. sonneratii*, 17 nucleotides substitutions (16 transitions and 1 transversions) and in *G. varius*, 24 nucleotide substitutions (21 transitions and 3 transversions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, 7 were fixed in these jungle fowls and all were transitions.

For ND4, in *G. lafayetei*, 72 nucleotide substitutions (65 transitions and 7 transversions), in *G. sonneratii*, 56 nucleotides substitutions (50 transitions and 6 transversions) and in *G. varius*, 76 nucleotide substitutions (68 transitions and 8 transversions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, 23 were fixed in these jungle fowls, out of which 20 were transitions and 3 was transversion..

For ND4L, in *G. lafayetei*, 9 nucleotide substitutions (all transitions), in *G. sonneratii*, 13 nucleotides substitutions (12 transitions and 1 transversions) and in *G. varius*, 6 nucleotide substitutions (all transitions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, only 1 was fixed in these jungle fowls and it was a transitions. For

ND5, in *G. lafayetei*, 87 nucleotide substitutions (76 transitions and 11 transversions), in *G. sonneratii*, 101 nucleotides substitutions (90 transitions and 11 transversions) and in *G. varius*, 44 nucleotide substitutions (40 transitions and 4 transversions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, 12 were fixed in these jungle fowls, out of which 9 were transitions and 3 was transversion. For ND6, in *G. lafayetei*, 36 nucleotide substitutions (32 transitions and 4 transversions), in *G. sonneratii*, 32 nucleotides substitutions (31 transitions and 1 transversions) and in *G. varius*, 42 nucleotide substitutions (37 transitions and 5 transversions) were found in comparison to other jungle fowl species. Among these nucleotide substitutions, 15 were fixed in these jungle fowls and all were transitions.

D. Genetic distances *G. gallus* subspecies and *Gallus* species

The genetic distances was estimated between different chicken breeds, red jungle fowl subspecies and jungle fowls as Jukes-Cantor genetic distance, based on transitions as well as transversions and are presented in Table 69 to 86 .

12S- ribosomal RNA gene

The Jukes-Cantor genetic distance were quite low between chicken breeds and ranged from ranged from 0.000 to 0.002. Sequence comparisons between groups reflected a very low genetic distance between domestic fowl and other red jungle fowl subspecies (0.000-0.003) in comparison to the other jungle fowls (0.032-0.037). Between the jungle fowls, more or less similar genetic distances were observed (0.029-0.040).

16S- ribosomal RNA gene

The Jukes-Cantor genetic distance were quite low between chicken breeds and ranged from ranged from 0.000 to 0.002. Between groups comparisons revealed very low genetic distance of red jungle fowl sub species with domestic fowl (0.001) in comparison to other jungle fowls (0.017-

0.036). The genetic distances between jungle fowls were more or less of similar magnitude (0.016-0.036).

ATPase genes

The Jukes-Cantor genetic distance were quite low between chicken breeds and ranged from ranged from 0.000 to 0.004 for ATPase6 gene and was absolutely zero for ATPase8 gene. Between the domestic fowl and jungle fowl, domestic fowl showed very low genetic distance with red jungle fowls (0.002-0.003) as compared to other jungle fowl (0.032-0.044) for ATP6, while for ATP8, there was complete sequence homology between domestic and red jungle fowl, while the genetic distances between domestic fowl and other jungle fowls ranged from 0.050-0.076. Between the jungle fowls, the genetic distances ranged from 0.030 to 0.048 for ATP6 and from 0.031 to 0.076 for ATP8.

Cytochrome oxidase genes

The genetic distances between different chicken breeds, based on nucleotide diversity in cytochrome oxidase genes were also quite low and ranged from 0.000 to 0.004, 0.000 to 0.003 and 0.000 to 0.004 for COI, COII and COIII, respectively. The domestic fowl showed least genetic distances with red jungle fowl (0.000 – 0.004) in comparison to other jungle fowls (0.021-0.067) for all the COX genes. Between the jungle fowls, the genetic distances ranged from 0.019 to 0.056 for COI, 0.036 to 0.046 for COII and 0.049 to 0.067 for COIII.

Cytochrome B

The Jukes-Cantor genetic distance were quite low between chicken breeds and ranged from ranged from 0.000 to 0.004. Between the domestic fowl and jungle fowl, domestic fowl showed very low genetic distance with red jungle fowls (0.003) as compared to other jungle fowl (0.040-0.056). Between the jungle fowls, the genetic distances ranged from 0.021 to 0.065.

NADH dehydrogenase genes

Between the chicken breeds the estimates of genetic distances were quite low for all the NADH dehydrogenase genes and ranged from 0.000 to 0.001,0.000 to 0.002, 0.000 to 0.006, 0.000 to 0.004, 0.000 to 0.003, 0.000 to 0.001 and 0.000 to 0.008 for ND1, ND2, ND3, ND4, ND4L, ND5 and ND6, respectively.

Domestic fowl invariably showed very low genetic distance with red jungle fowls (0.000 – 0.009) for all the ND genes, in comparison to other jungle fowls (0.021 – 0.072). Between the jungle fowls, the genetic distances ranged from 0.020 to 0.066 for ND1, 0.022 to 0.053 for ND2, 0.047 to 0.072 for ND3, 0.042 to 0.060 for ND4, 0.020 to 0.063 for ND4L, 0.024 to 0.063 for ND5 and 0.049 to 0.085 for ND6.

E. Phylogenetic between Gallus gallus subspecies and Gallus species

The genus *Gallus* is composed of four species i.e. *G. gallus*, *G. varius*, *G. lafayettei* and *G. sonneratii*. No consistent trend could be observed from phylogenetic trees drawn for different genes between these four jungle fowls, however the very close relatedness between different *G. gallus* subspecies, including *G. g. domesticus* was very much distinct as well as consistent for all the genes. Hence, phylogenetic analysis was made on cumulative nucleotide sequence variability for all the genes mentioned above (Fig 23). Among the different jungle fowls, *G. sonneratii* seemed to be separated out with other with the greatest distance. The second in remoteness was G. lafayattei, while the *G. varius* was comparatively close to *G. gallus*. These branching had very high bootstrap support (70 – 100 %). The different subspecies of *G. gallus* formed two clusters with 100 % bootstrap support. While the one cluster includes exclusively *G. g. domesticus*, other cluster include *G. g. gallus*, *G. g. bankiva* and *G. g. spadicus* along with two *G. g. domesticus* breeds i.e. White leghorn and Silky.

IIB. mt DNA polymorphism between *G. g. murghi*, Other *G. gallus* subspecies and *Gallus* species

A. Amplification of mitochondrial genes

16S-rRNA

The universal primers were used to amplify a ~ 590 bp 16S rRNA fragment from the genomic DNA extracted from Indian red jungle fowl. Using the PCR amplification conditions, ~ 590 bp fragment was successfully amplified. On resolution on 1.4 % agarose gel, presence of a single discrete band of ~ 590 bp showed the specificity of amplification (Fig 24).

12S-rRNA

The universal primer were used to amplify a ~ 440 bp 12S rRNA fragment from the genomic DNA extracted from the Indian red jungle fowl. Using the PCR amplification conditions, ~ 440 bp fragment was successfully amplified. On resolution on 1.4 % agarose gel, presence of a single discrete band of ~ 440 bp showed the specificity of amplification (Fig 25).

B. Cloning of mitochondrial genes

16S-rRNA

The ~590 bp 16S-rRNA fragment was cloned in p-GEMT Vector (Promega) using Ampicillin as selective antibiotic. Successful transfection was reflected by growth of typical white colonies of *E.Coli*. The plasmid was isolated using QIAprep miniprep kit (Qiagen), and resolved on 1.0 % agarose. The successful cloning of 590 bp 16S-rRNA fragment was then confirmed by restriction enzyme digestion with EcoRI restriction enzyme. The digested product was resolved on 1.4 % agarose gel and presence of a 3015 bp band representing vector and ~ 590 bp band representing 16S-rRNA fragment confirmed the successful cloning of the desired fragment.

12S-rRNA

Similarly, ~440 bp 12S-rRNA fragment was cloned in p-GEMT Vector (Promega). The successful cloning of 440 bp 12S-rRNA fragment was then

confirmed by restriction enzyme digestion with EcoRI restriction enzyme. The digested product was resolved on 1.4 % agarose gel and presence of a DNA of around 3015 bp band representing vector and ~ 440 bp band representing 12S-rRNA fragment confirmed the successful cloning of the desired fragment.

C. Sequencing of mitochondrial genes

The cloned amplified products of 16S rRNA as well as 12S rRNA were submitted for sequencing to Bangalore Genei, Bangalore. The sequencing was done using ABI Prism 377 DNA sequencer. The nucleotide sequences have been submitted to EMBL (European Molecular Biology Laboratory).

D. Nucleotide diversity between G. g. murghi, other G. gallus subspecies and Gallus species

12S rRNA

Alignment of the comparable portion of 12S rRNA gene from other domestic fowl breeds, *G. gallus* subspecies and *Gallus* species totaled 452 nucleotides positions (Fig 26). Between *G. g. murghi* and other subspecies i.e. *G. g. gallus*, *G. g. spadicus* and *G. g. bankiva* including *G. g. domesticus*, there was one insertion of C and two nucleotide substitutions (G by C and T by C) in *G. g. murghi*. However between *G. g. murghi* and other jungle fowls, there was insertion of 2 nucleotide in *G. lafayetei*, insertions of 3 nucleotides in *G. sonneratii* and 1 deltion in *G. varius*. Between G. g. murghi and *G. lafayetei*, a total of 15 nucleotides substitutions and 14 of these were transitions, while 1 was transversions. Between G. g. murghi and *G. sonneratii*, a total of 15 nucleotides substitutions and all of them were transitions. Similarly, between G. g. murghi and *G. varius*, a total of 14 nucleotides substitutions and 12 of these were transitions, while 2 were transversions. The *G. g. murghi* showed very low genetic distance with the domestic fowl breeds as well as with other *G. gallus* subspecies as the Jukes-Cantor genetic distance estimates ranged from 0.005 to 0.007, however the genetic

distances between *G. g. murghi* and other three jungle fowls were high and ranged from 0.035 to 0.042.

16 S RNA

Alignment of the comparable portion of 16S rRNA gene from other domestic fowl breeds, *G. gallus* subspecies and *Gallus* species totaled 612 nucleotides positions (Fig 27). Between *G. g. murghi* and other subspecies i.e. *G. g. gallus*, *G. g. spadicus* and *G. g. bankiva* including *G. g. domesticus*, two nucleotide substitutions (A by C and C by A) in *G. g. murghi*. However between *G. g. murghi* and other jungle fowl species, there was one 1 insertion and 1 deletion was found in *G. varius*. Between G. g. murghi and *G. lafayetei*, a total of 9 nucleotides substitutions and 8 of these were transitions, while 1 was transversions. Between G. g. murghi and *G. sonneratii*, a total of 19 nucleotides substitutions and 18 of them were transitions, while 1 was transversion. Similarly, between G. g. murghi and *G. varius*, a total of 8 nucleotides substitutions and 7 of these were transitions, while 1 was transversions. The Jukes-Cantor genetic distances also reflected the similar trend, as these estimates between *G. g. murghi* and other *G. gallus* subspecies including *G. g. domesticus* were 0.000 to 0.003. However, *G. g. murghi* showed distinct difference from other jungle fowls as even at this very low level of genetic distances, the Jukes-Cantor distances between them were 0.015 to 0.029.

Phylogenetic relationship

The phylogenetic analysis by Neighbour Joining method (Consensus tree based on 100 bootstrap replications) based on cumulative nucleotide sequence variation (Fig 28) showed that *G. g. murghi* made one cluster along with domestic fowl breeds and other *G. gallus* subspecies with a good bootstrap support (> 60 %). Other jungle fowls made separate groups. These results showed that *G. g. murghi* is also very close to its other allies as well as the domestic chicken breeds.

Table 3. Number of alleles (N), allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at ADL 268 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	5	106-116	0.472	0.200	0.484
WL	6	106-120	0.707	0.267	0.729
KN	7	100-120	0.785	0.400	0.800
AS	7	100-120	0.757	0.400	0.773
RC	7	100-120	0.791	0.866	0.804
Overall	10	100-120	0.862	0.427	0.869

Table 4. Number of alleles, allelic size range, polymorphic information content. (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 111 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	4	98-104	0.689	0.636	0.715
WL	5	98-108	0.385	0.231	0.393
KN	5	100-108	0.711	0.533	0.733
AS	4	100-106	0.528	0.286	0.556
RC	4	102-108	0.657	0.500	0.686
Overall	6	98-108	0.725	0.433	0.743

Table 5. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 248 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	6	212-232	0.748	0.400	0.764
WL	2	212-214	0.128	0.000	0.133
KN	5	210-228	0.756	0.400	0.773
AS	6	216-232	0.729	0.500	0.747
RC	6	216-232	0.752	0.666	0.771
Overall	12	210-238	0.876	0.397	0.881

Table 6. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 34 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	12	230-270	0.788	0.933	0.858
WL	6	232-256	0.716	0.800	0.802
KN	14	232-272	0.899	0.933	0.904
AS	12	232-270	0.796	1.000	0.882
RC	9	232-272	0.732	0.866	0.816
Overall	18	230-272	0.897	0.907	0.922

Table 7. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 37 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	5	156-178	0.731	0.143	0.753
WL	4	170-176	0.679	0.000	0.704
KN	8	144-178	0.838	0.133	0.847
AS	7	146-170	0.731	0.200	0.751
RC	6	144-156	0.775	0.333	0.791
Overall	13	144-178	0.907	0.164	0.910

Table 8. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 206 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	3	220-242	0.418	0.071	0.457
WL	6	206-226	0.637	0.077	0.654
KN	7	210-242	0.797	0.143	0.809
AS	6	206-230	0.810	0.400	0.822
RC	5	212-230	0.618	0.285	0.640
Overall	10	206-242	0.837	0.200	0.845

Table 9. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 222 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	2	230-234	0.495	0.000	0.486
WL	2	230-234	0.536	0.000	0.473
KN	5	220-254	0.653	0.733	0.589
AS	4	220-254	0.353	0.733	0.433
RC	1	234	0.353	0.000	0.000
Overall	5	220-254	0.803	0.310	0.591

Table 10. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 98 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	4	252-274	0.495	0.000	0.514
WL	3	264-274	0.536	0.000	0.571
KN	5	242-268	0.653	0.000	0.673
AS	2	242-248	0.353	0.000	0.391
RC	2	248-252	0.353	0.000	0.391
Overall	6	242-274	0.803	0.000	0.816

Table 11. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 284 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	5	372-424	0.727	0.364	0.748
WL	5	404-424	0.754	0.091	0.773
KN	3	400-414	0.573	0.000	0.611
AS	3	390-418	0.259	0.077	0.269
RC	3	390-426	0.581	0.400	0.620
Overall	11	372-428	0.872	0.191	0.878

Table 12. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 295 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	3	94-106	0.589	0.467	0.624
WL	8	90-114	0.776	0.667	0.789
KN	7	92-114	0.790	0.600	0.804
AS	5	98-110	0.739	0.200	0.758
RC	2	98-110	0.172	0.200	0.180
Overall	12	90-114	0.841	0.427	0.849

Table 13. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 81 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	6	90-146	0.584	0.429	0.597
WL	5	110-136	0.678	0.933	0.702
KN	6	110-146	0.702	0.714	0.724
AS	2	110-126	0.258	0.200	0.278
RC	2	110-126	0.121	0.133	0.124
Overall	7	110-146	0.732	0.480	0.752

Table 14. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at LEI 192 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	8	352-468	0.797	0.733	0.840
WL	7	218-276	0.772	0.400	0.833
KN	8	222-318	0.570	0.267	0.747
AS	6	252-352	0.592	0.467	0.727
RC	6	246-358	0.759	0.533	0.784
Overall	21	218-468	0.902	0.480	0.928

Table 15. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 14 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	8	182-206	0.840	0.667	0.849
WL	2	182-184	0.353	0.000	0.391
KN	8	168-190	0.803	0.571	0.814
AS	5	172-190	0.732	0.600	0.753
RC	4	170-186	0.559	0.200	0.589
Overall	15	168-206	0.872	0.405	0.878

Table 16. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 183 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	10	268-328	0.773	0.571	0.814
WL	4	268-308	0.528	0.467	0.713
KN	7	276-312	0.742	0.133	0.760
AS	14	278-328	0.882	0.733	0.889
RC	6	282-308	0.532	0.200	0.551
Overall	19	268-328	0.886	0.419	0.901

Table 17. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 78 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	4	146-152	0.608	0.200	0.633
WL	4	140-144	0.594	0.133	0.622
KN	6	140-150	0.739	0.067	0.758
AS	2	146-152	0.121	0.133	0.124
RC	5	140-152	0.721	0.466	0.740
Overall	7	140-152	0.789	0.200	0.801

Table 18. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 278 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	6	114-136	0.788	0.154	0.802
WL	4	118-136	0.485	0.133	0.507
KN	6	114-130	0.791	0.429	0.804
AS	3	114-122	0.313	0.333	0.331
RC	3	114-122	0.413	0.142	0.439
Overall	7	114-136	0.738	0.239	0.753

Table 19. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at ADL 112 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	2	130-132	0.357	0.000	0.397
WL	4	124-130	0.684	0.000	0.710
KN	5	124-134	0.718	0.133	0.738
AS	4	122-130	0.443	0.000	0.459
RC	5	122-130	0.712	0.000	0.735
Overall	7	122-134	0.770	0.030	0.785

Table 20. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 67 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	8	176-200	0.751	0.467	0.764
WL	6	170-196	0.685	0.429	0.704
KN	8	170-196	0.810	0.267	0.822
AS	8	172-198	0.804	0.733	0.816
RC	6	174-200	0.716	0.461	0.734
Overall	14	170-200	0.903	0.472	0.906

Table 21. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 216 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	3	156-160	0.471	0.133	0.498
WL	1	160	0.000	0.000	0.000
KN	4	144-160	0.645	0.133	0.678
AS	3	142-156	0.368	0.267	0.384
RC	2	142-144	0.431	0.200	0.491
Overall	6	142-160	0.747	0.147	0.763

Table 22. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 330 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	9	238-294	0.832	0.500	0.852
WL	9	234-286	0.803	0.400	0.820
KN	6	254-300	0.737	0.533	0.764
AS	8	262-300	0.782	0.400	0.796
RC	7	262-300	0.762	0.333	0.778
Overall	17	234-300	0.906	0.432	0.912

Table 23. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at MCW 69 locus in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	7	160-180	0.697	0.600	0.713
WL	9	154-180	0.600	0.333	0.611
KN	8	154-180	0.804	0.600	0.816
AS	8	154-180	0.767	0.400	0.782
RC	6	154-178	0.612	0.636	0.629
Overall	11	154-180	0.836	0.507	0.844

Table 24. Number of alleles, allelic size range, polymorphic information content (PIC), observed (Ho) and expected (Ht) heterozygocity at all the loci in different populations

Population	N	Allelic size (bp)	PIC	Ho	Ht
RJF	5.7	94-424	0.650	0.365	0.674
WL	4.8	90-424	0.573	0.255	0.602
KN	6.6	92-414	0.739	0.368	0.760
AS	5.7	98-418	0.577	0.384	0.606
RC	4.6	98-426	0.577	0.353	0.585
Overall	11.1	90-426	0.834	0.346	0.835

Table 25. Frequencies of different alleles for microsatellite locus ADL 268 in RJF and different chicken breeds

Alleles (bp)	Allelic Frequency				
	RJF	WL	KN	AS	RC
100	0.00	0.00	0.00	0.03	0.30
104	0.00	0.00	0.17	0.00	0.13
106	0.70	0.07	0.00	0.17	0.03
108	0.10	0.10	0.23	0.07	0.13
110	0.07	0.33	0.00	0.00	0.00
112	0.10	0.37	0.20	0.33	0.00
114	0.00	0.00	0.27	0.27	0.00
116	0.03	0.03	0.03	0.10	0.10
118	0.00	0.00	0.03	0.00	0.23
120	0.00	0.10	0.07	0.03	0.07
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 26. Frequencies of different alleles for microsatellite locus MCW 111 in RJF and different chicken breeds

Alleles (bp)	Allelic Frequency				
	RJF	WL	KN	AS	RC
98	0.36	0.04	0.00	0.00	0.00
100	0.32	0.77	0.30	0.61	0.00
102	0.14	0.08	0.37	0.25	0.39
104	0.18	0.04	0.07	0.11	0.36
106	0.00	0.00	0.17	0.04	0.14
108	0.00	0.08	0.10	0.00	0.11
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 27. Frequencies of different alleles for microsatellite locus MCW 248 in RJF and different chicken breeds

Alleles (bp)	Allelic Frequency				
	RJF	WL	KN	AS	RC
210	0.00	0.00	0.13	0.00	0.00
212	0.00	0.93	0.13	0.00	0.00
214	0.17	0.07	0.10	0.00	0.00
216	0.23	0.00	0.00	0.00	0.23
218	0.07	0.00	0.30	0.00	0.17
220	0.00	0.00	0.30	0.04	0.23
222	0.00	0.00	0.00	0.18	0.00
224	0.37	0.00	0.00	0.00	0.03
228	0.07	0.00	0.03	0.39	0.30
230	0.00	0.00	0.00	0.18	0.00
232	0.10	0.00	0.00	0.04	0.03
238	0.00	0.00	0.00	0.18	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 28. Frequencies of different alleles for microsatellite locus MCW 34 in RJF and different chicken breeds

Alleles (bp)	Allelic Frequency				
	RJF	WL	KN	AS	RC
230	0.23	0.00	0.00	0.00	0.00
232	0.00	0.27	0.03	0.03	0.27
234	0.10	0.13	0.10	0.10	0.23
236	0.07	0.00	0.00	0.00	0.00
238	0.00	0.00	0.07	0.03	0.03
244	0.00	0.20	0.17	0.13	0.00
246	0.23	0.00	0.00	0.00	0.00
248	0.00	0.00	0.07	0.07	0.00
250	0.03	0.00	0.07	0.13	0.07
252	0.03	0.03	0.03	0.07	0.10
254	0.07	0.17	0.03	0.00	0.03
256	0.03	0.20	0.10	0.00	0.20
260	0.00	0.00	0.10	0.03	0.00
262	0.07	0.00	0.03	0.03	0.00
264	0.03	0.00	0.03	0.03	0.03
266	0.07	0.00	0.00	0.20	0.00
270	0.03	0.00	0.13	0.13	0.00
272	0.00	0.00	0.03	0.00	0.03
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 29. Frequencies of different alleles for microsatellite locus MCW 37 in RJF and different chicken breeds

Alleles (bp)	Allelic Frequency				
	RJF	WL	KN	AS	RC
144	0.00	0.00	0.10	0.00	0.23
146	0.00	0.00	0.13	0.20	0.27
150	0.00	0.00	0.13	0.33	0.13
152	0.00	0.00	0.00	0.30	0.10
154	0.00	0.00	0.00	0.07	0.23
156	0.29	0.00	0.07	0.03	0.03
160	0.00	0.00	0.27	0.03	0.00
166	0.25	0.00	0.07	0.00	0.00
170	0.00	0.21	0.13	0.03	0.00
172	0.14	0.14	0.00	0.00	0.00
174	0.29	0.43	0.00	0.00	0.00
176	0.00	0.21	0.00	0.00	0.00
178	0.04	0.00	0.10	0.00	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 30. Frequencies of different alleles for microsatellite locus MCW 206 in RJF and different chicken breeds

Alleles (bp)	Allelic Frequency				
	RJF	**WL**	**KN**	**AS**	**RC**
206	0.00	0.54	0.00	0.17	0.00
208	0.00	0.00	0.00	0.23	0.00
210	0.00	0.15	0.07	0.00	0.00
212	0.00	0.08	0.14	0.20	0.04
216	0.00	0.00	0.00	0.10	0.07
218	0.00	0.04	0.32	0.00	0.00
220	0.29	0.15	0.14	0.17	0.54
226	0.68	0.04	0.18	0.00	0.14
230	0.00	0.00	0.07	0.13	0.21
242	0.04	0.00	0.07	0.00	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 31. Frequencies of different alleles for microsatellite locus MCW 222 in RJF and different chicken breeds

Alleles (bp)	Allelic Frequency				
	RJF	**WL**	**KN**	**AS**	**RC**
220	0.00	0.00	0.13	0.13	0.00
226	0.00	0.00	0.17	0.00	0.00
230	0.58	0.62	0.60	0.73	0.00
234	0.42	0.38	0.07	0.10	1.00
254	0.00	0.00	0.03	0.03	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 32. Frequencies of different alleles for microsatellite locus MCW 98 in RJF and different chicken breeds

Alleles (bp)	Allelic Frequency				
	RJF	WL	KN	AS	RC
242	0.00	0.00	0.14	0.27	0.00
248	0.00	0.00	0.21	0.73	0.27
252	0.17	0.00	0.07	0.00	0.73
264	0.08	0.29	0.50	0.00	0.00
268	0.08	0.57	0.07	0.00	0.00
274	0.67	0.14	0.00	0.00	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 33. Frequencies of different alleles for microsatellite locus MCW 284 in RJF and different chicken breeds

Alleles (bp)	Allelic Frequency				
	RJF	WL	KN	AS	RC
372	0.05	0.00	0.00	0.00	0.00
390	0.00	0.00	0.00	0.12	0.37
394	0.00	0.00	0.00	0.00	0.47
400	0.00	0.00	0.17	0.85	0.00
404	0.00	0.09	0.33	0.00	0.00
414	0.00	0.18	0.50	0.00	0.00
416	0.23	0.23	0.00	0.00	0.00
418	0.00	0.18	0.00	0.04	0.00
424	0.36	0.32	0.00	0.00	0.00
426	0.18	0.00	0.00	0.00	0.17
428	0.18	0.00	0.00	0.00	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 34. Frequencies of different alleles for microsatellite locus MCW 295 in RJF and different chicken breeds

Alleles (bp)	Allelic Frequency				
	RJF	WL	KN	AS	RC
140	0.00	0.03	0.00	0.00	0.00
142	0.00	0.03	0.23	0.00	0.00
144	0.50	0.00	0.00	0.00	0.00
146	0.00	0.17	0.27	0.00	0.00
148	0.27	0.00	0.10	0.20	0.90
150	0.00	0.00	0.00	0.10	0.00
152	0.00	0.00	0.13	0.00	0.00
154	0.00	0.37	0.00	0.00	0.00
156	0.23	0.13	0.20	0.20	0.00
158	0.00	0.13	0.00	0.13	0.00
160	0.00	0.03	0.03	0.37	0.10
164	0.00	0.10	0.03	0.00	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 35. Frequencies of different alleles for microsatellite locus MCW 81 in RJF and different chicken breeds

Allele (bp)	Allelic Frequency				
	RJF	WL	KN	AS	RC
110	0.04	0.27	0.29	0.17	0.93
112	0.11	0.17	0.18	0.00	0.00
122	0.00	0.03	0.07	0.00	0.00
126	0.07	0.43	0.00	0.83	0.07
128	0.61	0.00	0.39	0.00	0.00
136	0.11	0.10	0.04	0.00	0.00
146	0.07	0.00	0.04	0.00	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 36. Frequencies of different alleles for microsatellite locus LEI 192 in RJF and different chicken breeds

Allele (bp)	Allelic Frequency				
	RJF	WL	KN	AS	RC
218	0.00	0.10	0.00	0.00	0.00
222	0.00	0.20	0.40	0.00	0.00
232	0.00	0.20	0.00	0.00	0.00
236	0.00	0.13	0.07	0.00	0.00
246	0.00	0.20	0.27	0.00	0.30
252	0.00	0.00	0.03	0.07	0.27
264	0.00	0.00	0.03	0.00	0.17
272	0.00	0.13	0.00	0.00	0.00
276	0.00	0.03	0.07	0.00	0.00
282	0.00	0.00	0.03	0.13	0.00
304	0.00	0.00	0.00	0.37	0.00
318	0.00	0.00	0.10	0.07	0.00
326	0.00	0.00	0.00	0.33	0.07
352	0.07	0.00	0.00	0.03	0.07
358	0.07	0.00	0.00	0.00	0.13
368	0.10	0.00	0.00	0.00	0.00
400	0.23	0.00	0.00	0.00	0.00
426	0.13	0.00	0.00	0.00	0.00
444	0.23	0.00	0.00	0.00	0.00
452	0.07	0.00	0.00	0.00	0.00
468	0.10	0.00	0.00	0.00	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 37. Frequencies of different alleles for microsatellite locus MCW 14 in RJF and different chicken breeds

Allele (bp)	Allelic Frequency				
	RJF	**WL**	**KN**	**AS**	**RC**
168	0.00	0.00	0.11	0.00	0.00
170	0.00	0.00	0.18	0.00	0.27
172	0.00	0.00	0.04	0.20	0.57
174	0.00	0.00	0.00	0.20	0.00
176	0.00	0.00	0.32	0.23	0.00
180	0.00	0.00	0.07	0.00	0.00
182	0.17	0.73	0.14	0.00	0.00
184	0.23	0.27	0.00	0.00	0.13
186	0.00	0.00	0.00	0.03	0.03
188	0.13	0.00	0.04	0.00	0.00
190	0.07	0.00	0.11	0.33	0.00
196	0.07	0.00	0.00	0.00	0.00
202	0.07	0.00	0.00	0.00	0.00
204	0.17	0.00	0.00	0.00	0.00
206	0.10	0.00	0.00	0.00	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 38. Frequencies of different alleles for microsatellite locus MCW 183 in RJF and different chicken breeds

Allele (bp)	Allelic Frequency				
	RJF	**WL**	**KN**	**AS**	**RC**
268	0.11	0.40	0.00	0.00	0.00
274	0.14	0.00	0.00	0.00	0.00
276	0.00	0.23	0.17	0.00	0.00
278	0.36	0.13	0.33	0.13	0.00
282	0.00	0.00	0.00	0.20	0.07
286	0.00	0.00	0.00	0.07	0.63
288	0.07	0.00	0.07	0.03	0.20
294	0.04	0.00	0.07	0.03	0.00
296	0.00	0.00	0.30	0.03	0.03
302	0.07	0.00	0.00	0.13	0.03
308	0.11	0.23	0.03	0.00	0.03
312	0.00	0.00	0.03	0.03	0.00
314	0.04	0.00	0.00	0.03	0.00
318	0.00	0.00	0.00	0.13	0.00
320	0.00	0.00	0.00	0.07	0.00
322	0.00	0.00	0.00	0.03	0.00
326	0.04	0.00	0.00	0.03	0.00
328	0.04	0.00	0.00	0.03	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 39. Frequencies of different alleles for microsatellite locus MCW 78 in RJF and different chicken breeds

Allele (bp)	Allelic Frequency				
	RJF	WL	KN	AS	RC
140	0.00	0.13	0.13	0.00	0.23
142	0.00	0.53	0.30	0.00	0.00
144	0.00	0.27	0.07	0.00	0.40
146	0.23	0.07	0.33	0.93	0.13
148	0.13	0.00	0.13	0.00	0.00
150	0.53	0.00	0.03	0.00	0.10
152	0.10	0.00	0.00	0.07	0.13
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 40. Frequencies of different alleles for microsatellite locus ADL 278 in RJF and different chicken breeds

Allele (bp)	Allelic Frequency				
	RJF	WL	KN	AS	RC
114	0.08	0.00	0.14	0.17	0.07
118	0.15	0.20	0.14	0.00	0.00
120	0.19	0.00	0.32	0.80	0.71
122	0.00	0.00	0.11	0.03	0.21
126	0.15	0.07	0.14	0.00	0.00
130	0.31	0.67	0.14	0.00	0.00
136	0.12	0.07	0.00	0.00	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 41. Frequencies of different alleles for microsatellite locus ADL 112 in RJF and different chicken breeds

Allele (bp)	Allelic Frequency				
	RJF	WL	KN	AS	RC
122	0.00	0.00	0.00	0.14	0.21
124	0.00	0.31	0.20	0.07	0.36
126	0.00	0.38	0.20	0.71	0.29
128	0.00	0.15	0.40	0.00	0.07
130	0.73	0.15	0.13	0.07	0.07
132	0.27	0.00	0.00	0.00	0.00
134	0.00	0.00	0.07	0.00	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 42. Frequencies of different alleles for microsatellite locus MCW 67 in RJF and different chicken breeds

Allele (bp)	Allelic Frequency				
	RJF	**WL**	**KN**	**AS**	**RC**
170	0.00	0.46	0.23	0.00	0.00
172	0.00	0.21	0.23	0.20	0.00
174	0.00	0.11	0.00	0.30	0.08
176	0.03	0.00	0.13	0.03	0.19
178	0.07	0.00	0.07	0.07	0.42
180	0.10	0.00	0.20	0.10	0.08
184	0.40	0.00	0.00	0.00	0.00
186	0.20	0.00	0.03	0.00	0.00
188	0.00	0.14	0.03	0.00	0.00
192	0.00	0.00	0.00	0.03	0.19
194	0.00	0.04	0.00	0.00	0.00
196	0.03	0.04	0.07	0.17	0.00
198	0.03	0.00	0.00	0.10	0.00
200	0.13	0.00	0.00	0.00	0.04
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 43. Frequencies of different alleles for microsatellite locus MCW 216 in RJF and different chicken breeds

Allele (bp)	Allelic Frequency				
	RJF	**WL**	**KN**	**AS**	**RC**
142	0.00	0.00	0.00	0.10	0.57
144	0.00	0.00	0.40	0.00	0.43
146	0.00	0.00	0.03	0.77	0.00
156	0.20	0.00	0.30	0.13	0.00
158	0.13	0.00	0.00	0.00	0.00
160	0.67	1.00	0.27	0.00	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 44. Frequencies of different alleles for microsatellite locus MCW 330 in RJF and different chicken breeds

Allele (bp)	Allelic Frequency				
	RJF	**WL**	**KN**	**AS**	**RC**
234	0.00	0.03	0.00	0.00	0.00
238	0.04	0.07	0.00	0.00	0.00
242	0.00	0.17	0.00	0.00	0.00
250	0.25	0.00	0.00	0.00	0.00
254	0.11	0.23	0.33	0.00	0.00
258	0.14	0.03	0.00	0.00	0.00
262	0.00	0.00	0.00	0.17	0.10
266	0.00	0.00	0.00	0.00	0.33
268	0.14	0.03	0.00	0.07	0.13
270	0.00	0.27	0.00	0.37	0.27
274	0.14	0.00	0.00	0.07	0.00
276	0.00	0.03	0.27	0.00	0.00
278	0.07	0.00	0.07	0.13	0.00
286	0.04	0.13	0.20	0.00	0.03
292	0.00	0.00	0.00	0.07	0.10
294	0.07	0.00	0.07	0.03	0.00
300	0.00	0.00	0.07	0.10	0.03
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 45. Frequencies of different alleles for microsatellite locus MCW 69 in RJF and different chicken breeds

Allele (bp)	Allelic Frequency				
	RJF	**WL**	**KN**	**AS**	**RC**
154	0.00	0.03	0.10	0.03	0.57
156	0.00	0.03	0.10	0.00	0.10
158	0.00	0.03	0.03	0.00	0.10
160	0.03	0.03	0.17	0.10	0.00
162	0.20	0.60	0.30	0.33	0.00
164	0.47	0.13	0.00	0.27	0.00
172	0.00	0.00	0.03	0.03	0.17
174	0.03	0.00	0.00	0.07	0.00
176	0.07	0.07	0.20	0.00	0.03
178	0.07	0.03	0.00	0.03	0.03
180	0.13	0.03	0.07	0.13	0.00
	1.00	**1.00**	**1.00**	**1.00**	**1.00**

Table 46. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at ADL 268 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.253**	0.585	0.299	0.375	0.174
WL	0.536	**0.211**	0.379	0.500	0.347
KN	1.207	0.970	**0.200**	0.429	0.428
AS	0.981	0.693	0.846	**0.200**	0.372
RC	1.749	1.058	0.849	0.989	**0.267**

Table 47. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 111 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.409**	0.408	0.344	0.556	0.286
WL	0.896	**0.246**	0.482	0.354	0.266
KN	1.067	0.730	**0.306**	0.504	0.669
AS	0.587	1.038	0.685	**0.321**	0.419
RC	1.252	1.324	0.402	0.870	**0.375**

Table 48. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 248 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.244**	0.090	0.267	0.113	0.453
WL	2.408	**0.333**	0.174	0.000	0.000
KN	1.321	1.749	**0.233**	0.061	0.202
AS	2.180	-	2.797	**0.250**	0.259
RC	0.792	-	1.599	1.351	**0.278**

Table 49. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 34 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.161**	0.210	0.359	0.298	0.270
WL	1.561	**0.300**	0.315	0.218	0.337
KN	1.024	1.155	**0.138**	0.660	0.504
AS	1.211	1.523	0.416	**0.166**	0.318
RC	1.309	1.088	0.685	1.146	**0.207**

Table 50. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 37 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.228**	0.369	0.172	0.040	0.044
WL	0.997	**0.250**	0.082	0.057	0.000
KN	1.760	2.501	**0.142**	0.371	0.341
AS	3.219	2.865	0.992	**0.171**	0.470
RC	3.124	-	1.076	0.755	**0.222**

Table 51. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 206 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.381**	0.129	0.301	0.117	0.182
WL	2.048	**0.179**	0.414	0.239	0.286
KN	1.201	0.882	**0.183**	0.244	0.275
AS	2.146	1.431	1.411	**0.233**	0.380
RC	1.704	1.252	1.291	0.968	**0.257**

Table 52. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 222 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.500**	0.998	0.261	0.433	0.355
WL	0.002	**0.500**	0.267	0.446	0.335
KN	1.343	1.321	**0.226**	0.717	0.027
AS	0.837	0.807	0.333	**0.300**	0.096
RC	1.036	1.094	3.612	2.343	**1.000**

Table 53. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 98 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.250**	0.308	0.339	0.000	0.086
WL	1.178	**0.333**	0.185	0.000	0.000
KN	1.082	1.687	**0.200**	0.274	0.234
AS	-	-	1.295	**0.500**	0.214
RC	2.453	-	1.452	1.542	**0.500**

Table 54. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 284 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.341**	0.247	0.000	0.000	0.142
WL	1.398	**0.236**	0.192	0.065	0.000
KN	-	1.650	**0.333**	0.076	0.000
AS	-	2.733	2.577	**0.359**	0.134
RC	1.952	-	-	2.010	**0.488**

Table 55. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 295 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.489**	0.053	0.176	0.246	0.138
WL	2.937	**0.208**	0.357	0.194	0.067
KN	1.737	1.030	**0.228**	0.180	0.123
AS	1.402	1.640	1.715	**0.306**	0.258
RC	1.981	2.703	2.096	1.355	**0.600**

Table 56. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 81 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.238**	0.345	0.519	0.063	0.202
WL	1.064	**0.373**	0.481	0.383	0.207
KN	0.656	0.732	**0.285**	0.141	0.137
AS	2.765	0.960	1.959	**0.800**	0.564
RC	1.599	1.575	1.988	0.573	**0.366**

Table 57. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at LEI 192 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.225**	0.000	0.000	0.062	0.150
WL	-	**0.200**	0.304	0.000	0.083
KN	-	1.191	**0.166**	0.200	0.163
AS	2.781	-	1.609	**0.244**	0.208
RC	1.897	2.489	1.814	1.570	**0.256**

Table 58. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 14 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.208**	0.179	0.190	0.035	0.073
WL	1.720	**0.500**	0.075	0.000	0.192
KN	1.661	2.590	**0.196**	0.213	0.122
AS	3.352	-	1.546	**0.320**	0.249
RC	2.617	1.650	2.104	1.390	**0.300**

Table 59. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 183 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.157**	0.172	0.290	0.378	0.151
WL	1.760	**0.367**	0.242	0.059	0.031
KN	1.238	1.419	**0.178**	0.192	0.221
AS	0.973	2.830	1.650	**0.124**	0.200
RC	1.890	3.474	1.510	1.609	**0.200**

Table 60. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 78 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.333**	0.049	0.313	0.305	0.367
WL	3.016	**0.283**	0.482	0.052	0.441
KN	1.162	0.730	**0.189**	0.099	0.293
AS	1.187	2.957	2.313	**0.667**	0.177
RC	1.002	0.819	1.228	1.732	**0.367**

Table 61. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 278 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.205**	0.525	0.664	0.162	0.220
WL	0.644	**0.283**	0.349	0.000	0.000
KN	0.409	1.053	**0.238**	0.370	0.433
AS	1.820	-	0.994	**0.533**	0.552
RC	1.514	-	0.837	0.594	**0.381**

Table 62. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at ADL 112 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.500**	0.081	0.059	0.039	0.032
WL	2.513	**0.250**	0.663	0.473	0.695
KN	2.830	0.411	**0.226**	0.383	0.487
AS	3.244	0.749	0.960	**0.321**	0.476
RC	3.442	0.364	0.719	0.742	**0.200**

Table 63. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 67 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.175**	0.077	0.360	0.337	0.208
WL	2.564	**0.238**	0.276	0.174	0.076
KN	1.022	1.287	**0.158**	0.383	0.137
AS	1.088	1.749	0.960	**0.216**	0.242
RC	1.570	2.577	1.988	1.419	**0.243**

Table 64. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 216 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.378**	0.318	0.327	0.192	0.000
WL	1.146	**1.000**	0.150	0.000	0.000
KN	1.118	1.897	**0.283**	0.226	0.192
AS	1.650	-	1.487	**0.422**	0.096
RC	-	-	1.650	2.343	**0.600**

Table 65. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 330 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.167**	0.273	0.216	0.256	0.133
WL	1.298	**0.155**	0.184	0.166	0.175
KN	1.532	1.693	**0.255**	0.248	0.102
AS	1.363	1.796	1.394	**0.175**	0.438
RC	2.017	1.743	2.283	0.826	**0.190**

Table 66. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at MCW 69 in different populations

	RJF	WL	KN	AS	RC
RJF	**0.228**	0.440	0.271	0.557	0.145
WL	0.821	**0.141**	0.505	0.436	0.355
KN	1.306	0.683	**0.200**	0.399	0.287
AS	0.585	0.830	0.919	**0.175**	0.140
RC	1.931	1.036	1.248	1.966	**0.266**

Table 67. Within population genetic similarity (diagonal), between population genetic similarity (above diagonal) and between population genetic distance (below diagonal) based on band frequency at all the loci in different populations

	RJF	WL	KN	AS	RC
RJF	**0.289**	0.279	0.273	0.217	0.181
WL	1.277	**0.314**	0.312	0.182	0.185
KN	1.299	1.164	**0.217**	0.303	0.256
AS	1.526	1.705	1.193	**0.324**	0.298
RC	1.707	1.685	1.362	1.210	**0.360**

Table 68. Nucleotide variability in different mitochondrial genes between chicken breeds, between *G. gallus* subspecies and *Gallus* species.

Mt genes	Between chicken Breeds				Between *G. gallus* subspecies				Between *Gallus* species			
	Size	Nt substitutions			Size	Nt substitutions			Size	Nt substitutions		
		Ts	Tr	Total		TS	Tr	Total		Ts	Tr	Total
12S- rRNA	976	3	0	3	no size	1	1	2	973-978	30-34	1-2	31-36
16S-rRNA	1621-1626	3	0	3	1622	2	0	2	1618-1622	24-57	3-5	27-62
ATPase6	684	3	0	3	684	1	0	1	684	19-24	1-4	20-28
ATPase8	195	0	0	0	165	0	0	0	165	8-12	0	8-12
COI	1551	6	1	7	1551	1	0	1	1551	28-78	2-6	30-84
COII	684	2	1	3	684	1	0	1	684	24-26	1-3	25-28
COIII	784-787	3	0	3	786	2	0	2	784-786	36-42	4-7	42-47
CYTB	1143	2	0	2	1143	3	1	3	1143	36-51	4-8	43-59
ND1	975	1	0	1	975	2	0	2	975	19-51	0-4	19-55
ND2	1041	1	1	2	1041	1	1	2	1041	19-48	4-6	23-55
ND3	351	2	0	2	351	1	0	1	351	16-21	0-3	16-24
ND4	1378	6	0	6	1380	3	0	3	1378-1380	50-68	6-8	56-76
ND4L	297	1	0	1	297	2	1	3	297	6-12	0-1	6-13
ND5	1818	5	1	6	1818	1	0	1	1818	40-90	4-11	44-101
ND6	522	4	0	4	522	2	0	2	522	31-37	1-5	32-42

Table 69. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for 12S rRNA gene (below diagonal) and 16S rRNA (above diagonal) genes

	G. g. bankiva	*G. g. domesticus*	*G. g. gallus*	*G. g. spadicus*	*G. lafayettei*	*G.sonneratii*	*G. varius*
G. g. bankiva		0.001	0.000	0.001	0.029	0.035	0.016
G. g. domesticus	0.001		0.001	0.001	0.030	0.036	0.016
G. g. gallus	0.000	0.001		0.001	0.029	0.035	0.016
G. g. spadicus	0.002	0.003	0.002		0.030	0.036	0.017
G. lafayettei	0.037	0.037	0.037	0.038		0.025	0.026
G. sonneratii	0.035	0.035	0.035	0.037	0.040		0.029
G. varius	0.031	0.032	0.031	0.034	0.039	0.029	

Table 70. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for ATPase6 gene (below diagonal) and ATPase8 (above diagonal) genes

	G. g. bankiva	*G. g. domesticus*	*G. g. gallus*	*G. g. spadicus*	*G. lafayettei*	*G.sonneratii*	*G. varius*
G. g. bankiva		0.000	0.000	0.000	0.076	0.050	0.076
G. g. domesticus	0.002		0.000	0.000	0.076	0.050	0.070
G. g. gallus	0.000	0.002		0.000	0.076	0.050	0.076
G. g. spadicus	0.001	0.003	0.001		0.076	0.050	0.070
G. lafayettei	0.030	0.032	0.030	0.031		0.037	0.031
G. sonneratii	0.042	0.044	0.042	0.044	0.045		0.031
G. varius	0.036	0.038	0.036	0.037	0.036	0.048	

Table 71. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for Cytochrome Oxidase I (below diagonal) and Cytochrome Oxidase II (above diagonal) genes

	G. g. bankiva	*G. g. domesticus*	*G. g. gallus*	*G. g. spadicus*	*G. lafayettei*	*G.sonneratii*	*G. varius*
G. g. bankiva		0.004	0.000	0.001	0.039	0.037	0.045
G. g. domesticus	0.003		0.004	0.002	0.043	0.041	0.046
G. g. gallus	0.001	0.002		0.001	0.039	0.037	0.045
G. g. spadicus	0.001	0.002	0.000		0.041	0.039	0.044
G. lafayettei	0.035	0.036	0.034	0.034		0.036	0.045
G. sonneratii	0.055	0.056	0.056	0.056	0.046		0.055
G. varius	0.019	0.021	0.020	0.020	0.046	0.053	

Table 72. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for Cytochrome Oxidase III (below diagonal) and Cytochrome Oxidase B (above diagonal) genes

	G. g. bankiva	*G. g. domesticus*	*G. g. gallus*	*G. g. spadicus*	*G. lafayettei*	*G.sonneratii*	*G. varius*
G. g. bankiva		0.003	0.002	0.004	0.054	0.053	0.041
G. g. domesticus	0.003		0.003	0.003	0.056	0.053	0.040
G. g. gallus	0.001	0.001		0.002	0.057	0.053	0.065
G. g. spadicus	0.003	0.003	0.001		0.057	0.054	0.041
G. lafayettei	0.054	0.054	0.053	0.051		0.065	0.021
G. sonneratii	0.058	0.059	0.057	0.056	0.049		0.057
G. varius	0.067	0.067	0.065	0.064	0.053	0.050	

Table 73. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for NADH dehrogenase 1 (below diagonal) and NADH dehrogenase 2 (above diagonal) genes

	G. g. bankiva	*G. g. domesticus*	*G. g. gallus*	*G. g. spadicus*	*G. lafayettei*	*G.sonneratii*	*G. varius*
G. g. bankiva		0.001	0.000	0.002	0.053	0.051	0.022
G. g. domesticus	0.003		0.001	0.001	0.052	0.050	0.023
G. g. gallus	0.000	0.003		0.002	0.053	0.051	0.022
G. g. spadicus	0.002	0.001	0.002		0.053	0.051	0.024
G. lafayettei	0.056	0.053	0.056	0.054		0.040	0.052
G. sonneratii	0.061	0.059	0.061	0.059	0.055		0.043
G. varius	0.022	0.021	0.022	0.020	0.055	0.066	

Table 74. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for NADH dehydrogenase 3 (below diagonal) and NADH dehydrogenase 4 (above diagonal) genes

	G. g. bankiva	*G. g. domesticus*	*G. g. gallus*	*G. g. spadicus*	*G. lafayettei*	*G.sonneratii*	*G. varius*
G. g. bankiva		0.003	0.001	0.001	0.056	0.043	0.060
G. g. domesticus	0.002		0.003	0.002	0.054	0.043	0.059
G. g. gallus	0.000	0.002		0.001	0.056	0.043	0.060
G. g. spadicus	0.003	0.004	0.003		0.054	0.042	0.058
G. lafayettei	0.062	0.061	0.062	0.065		0.053	0.060
G. sonneratii	0.050	0.049	0.050	0.053	0.047		0.056
G. varius	0.065	0.064	0.065	0.069	0.072	0.056	

Table 75. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for NADH dehydrogenase 4L (below diagonal) and NADH dehydrogenase 5 (above diagonal) genes

	G. g. bankiva	*G. g. domesticus*	*G. g. gallus*	*G. g. spadicus*	*G. lafayettei*	*G.sonneratii*	*G. varius*
G. g. bankiva		0.001	0.001	0.001	0.049	0.057	0.025
G. g. domesticus	0.009		0.000	0.001	0.050	0.058	0.024
G. g. gallus	0.007	0.009		0.001	0.050	0.058	0.024
G. g. spadicus	0.007	0.002	0.007		0.051	0.058	0.025
G. lafayettei	0.038	0.033	0.038	0.031		0.059	0.059
G. sonneratii	0.052	0.047	0.052	0.045	0.063		0.063
G. varius	0.027	0.023	0.027	0.020	0.045	0.045	

Table 76. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for NADH dehydrogenase 6 (below diagonal) and NADH dehydrogenase 5 (above diagonal) genes

	G. g. bankiva	*G. g. domesticus*	*G. g. gallus*	*G. g. spadicus*	*G. lafayettei*	*G.sonneratii*	*G. varius*
G. g. bankiva							
G. g. domesticus	0.003						
G. g. gallus	0.002	0.001					
G. g. spadicus	0.004	0.002	0.002				
G. lafayettei	0.070	0.072	0.072	0.070			
G. sonneratii	0.062	0.064	0.064	0.062	0.049		
G. varius	0.083	0.085	0.085	0.083	0.070	0.072	

Table 77. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for 12S rRNA gene (below diagonal) and 16S rRNA (above diagonal) genes

	Silky	WLH-1	WPR	Native Laos	WLH-2	NH	Tibetian	WLH-3
Silky		0.001	0.001	0.001	0.001	0.001	0.001	0.001
WLH-1	0.002		0.000	0.000	0.000	0.000	0.000	0.002
WPR	0.001	0.001		0.000	0.000	0.000	0.000	0.002
Native Laos	0.001	0.001	0.000		0.000	0.000	0.000	0.002
WLH-2	0.001	0.001	0.000	0.000		0.000	0.000	0.002
NH	0.002	0.002	0.001	0.001	0.001		0.000	0.002
Tibetian	0.001	0.001	0.000	0.000	0.000	0.001		0.002
WLH-3	0.000	0.002	0.001	0.001	0.001	0.002	0.001	

Table 78. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for ATPase6 gene (below diagonal) and ATPase8 (above diagonal) genes

	Silky	WLH-1	WPR	Native Laos	WLH-2	NH	Tibetian	WLH-3
Silky		0.000	0.000	0.000	0.000	0.000	0.000	0.000
WLH-1	0.004		0.000	0.000	0.000	0.000	0.000	0.000
WPR	0.004	0.000		0.000	0.000	0.000	0.000	0.000
Native Laos	0.004	0.000	0.000		0.000	0.000	0.000	0.000
WLH-2	0.004	0.000	0.000	0.000		0.000	0.000	0.000
NH	0.004	0.000	0.000	0.000	0.000		0.000	0.000
Tibetian	0.004	0.000	0.000	0.000	0.000	0.000		0.000
WLH-3	0.000	0.004	0.004	0.004	0.004	0.004	0.004	

Table 79. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for Cytochrome Oxidase I (below diagonal) and Cytochrome Oxidase II (above diagonal) genes

	Silky	WLH-1	WPR	Native Laos	WLH-2	NH	Tibetian	WLH-3
Silky		0.003	0.003	0.003	0.003	0.003	0.003	0.000
WLH-1	0.003		0.000	0.000	0.000	0.000	0.003	0.003
WPR	0.003	0.000		0.000	0.000	0.000	0.003	0.003
Native Laos	0.003	0.000	0.000		0.000	0.000	0.003	0.003
WLH-2	0.003	0.000	0.000	0.000		0.000	0.003	0.003
NH	0.003	0.000	0.000	0.000	0.000		0.003	0.003
Tibetian	0.003	0.004	0.004	0.004	0.004	0.004		0.003
WLH-3	0.001	0.003	0.003	0.003	0.003	0.003	0.003	

Table 80. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for Cytochrome Oxidase III (below diagonal) and Cytochrome Oxidase B (above diagonal) genes

	Silky	WLH-1	WPR	Native Laos	WLH-2	NH	Tibetian	WLH-3
Silky		0.001	0.001	0.001	0.001	0.002	0.000	0.001
WLH-1	0.003		0.000	0.000	0.000	0.001	0.001	0.000
WPR	0.003	0.000		0.000	0.000	0.001	0.001	0.000
Native Laos	0.003	0.000	0.000		0.000	0.001	0.001	0.000
WLH-2	0.003	0.000	0.000	0.000		0.001	0.001	0.000
NH	0.003	0.000	0.000	0.000	0.000		0.002	0.001
Tibetian	0.004	0.001	0.001	0.001	0.001	0.001		0.001
WLH-3	0.000	0.003	0.003	0.003	0.003	0.003	0.004	

Table 81. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for NADH dehrogenase 1 (below diagonal) and NADH dehrogenase 2 (above diagonal) genes

	Silky	WLH-1	WPR	Native Laos	WLH-2	NH	Tibetian	WLH-3
Silky		0.001	0.001	0.001	0.001	0.001	0.001	0.002
WLH-1	0.001		0.000	0.000	0.000	0.000	0.000	0.001
WPR	0.001	0.000		0.000	0.000	0.000	0.000	0.001
Native Laos	0.001	0.000	0.000		0.000	0.000	0.000	0.001
WLH-2	0.001	0.000	0.000	0.000		0.000	0.000	0.001
NH	0.001	0.000	0.000	0.000	0.000		0.000	0.001
Tibetian	0.001	0.000	0.000	0.000	0.000	0.000		0.001
WLH-3	0.000	0.001	0.001	0.001	0.001	0.001	0.001	

Table 82. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for NADH dehydrogenase 3 (below diagonal) and NADH dehydrogenase 4 (above diagonal) genes

	Silky	WLH-1	WPR	Native Laos	WLH-2	NH	Tibetian	WLH-3
Silky		0.003	0.004	0.003	0.003	0.003	0.003	0.000
WLH-1	0.006		0.001	0.000	0.000	0.000	0.001	0.003
WPR	0.006	0.000		0.001	0.001	0.001	0.002	0.004
Native Laos	0.006	0.000	0.000		0.000	0.000	0.001	0.003
WLH-2	0.006	0.000	0.000	0.000		0.000	0.001	0.003
NH	0.006	0.000	0.000	0.000	0.000		0.001	0.003
Tibetian	0.003	0.003	0.003	0.003	0.003	0.003		0.003
WLH-3	0.000	0.006	0.006	0.006	0.006	0.006	0.003	

Table 83. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for NADH dehydrogenase 4L (below diagonal) and NADH dehydrogenase 5 (above diagonal) genes

	Silky	WLH-1	WPR	Native Laos	WLH-2	NH	Tibetian	WLH-3
Silky		0.001	0.001	0.001	0.001	0.001	0.001	0.001
WLH-1	0.003		0.001	0.001	0.001	0.000	0.001	0.001
WPR	0.003	0.000		0.001	0.001	0.001	0.001	0.001
Native Laos	0.003	0.000	0.000		0.001	0.001	0.001	0.001
WLH-2	0.003	0.000	0.000	0.000		0.001	0.001	0.001
NH	0.003	0.000	0.000	0.000	0.000		0.001	0.001
Tibetian	0.000	0.003	0.003	0.003	0.003	0.003		0.001
WLH-3	0.000	0.003	0.003	0.003	0.003	0.003	0.000	

Table 84. Nucleotide divergence (Jukes-Cantor genetic distance) between different species and subspecies under genus Gallus for NADH dehydrogenase 6

	Silky	WLH-1	WPR	Native Laos	WLH-2	NH	Tibetian	WLH-3
Silky								
WLH-1	0.006							
WPR	0.006	0.000						
Native Laos	0.006	0.000	0.000					
WLH-2	0.006	0.000	0.000	0.000				
NH	0.006	0.000	0.000	0.000	0.000			
Tibetian	0.008	0.002	0.002	0.002	0.002	0.002		
WLH-3	0.004	0.002	0.002	0.002	0.002	0.002	0.004	

Table 85. Pair-wise genetic distance (Jukes-Cantor) between different Gallus species and Gallus gallus subspecies for 448 nucleotide positions in 12S rRNA gene

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]
[1]	-																
[2]	0.007	-															
[3]	0.005	0.002	-														
[4]	0.007	0.005	0.002	-													
[5]	0.005	0.002	0.000	0.002	-												
[6]	0.005	0.002	0.000	0.002	0.000	-											
[7]	0.007	0.005	0.002	0.005	0.002	0.002	-										
[8]	0.005	0.002	0.000	0.002	0.000	0.000	0.002	-									
[9]	0.007	0.005	0.002	0.000	0.002	0.002	0.005	0.002	-								
[10]	0.005	0.002	0.000	0.002	0.000	0.000	0.002	0.000	0.002	-							
[11]	0.005	0.002	0.000	0.002	0.000	0.000	0.002	0.000	0.002	0.000	-						
[12]	0.005	0.002	0.000	0.002	0.000	0.000	0.002	0.000	0.002	0.000	0.000	-					
[13]	0.005	0.002	0.000	0.002	0.000	0.000	0.002	0.000	0.002	0.000	0.000	0.000	-				
[14]	0.042	0.035	0.037	0.040	0.037	0.037	0.040	0.037	0.040	0.037	0.037	0.037	0.037	-			
[15]	0.035	0.028	0.030	0.033	0.030	0.030	0.033	0.030	0.033	0.030	0.030	0.030	0.030	0.040	-		
[16]	0.035	0.028	0.030	0.033	0.030	0.030	0.033	0.030	0.033	0.030	0.030	0.030	0.030	0.040	0.028	-	
[17]	0.092	0.084	0.087	0.090	0.087	0.087	0.090	0.087	0.090	0.087	0.087	0.087	0.087	0.079	0.084	0.084	-

Note : **[1]**-G.g. murghi; **[2]**-G.g. domesticus (WLH); **[3]**-G.g. domesticus (WLH); **[4]** G.g. domesticus (WLH); **[5]** G.g.domesticus (WPR); **[6]** G.g.domesticus (NL); **[7]** G.g.domesticus (NH); **[8]**G.g. domesticus (T); **[9]** G.g.domesticus (SL); **[10]** G.g.gallus1; **[11]** G.g.gallus2; **[12]** G.g.bankiva; **[13]** G.g.spadicus; **[14]** G.lafayettei; **[15]** G.sonneratii; **[16]** G.varius; **[17]** N.meleagris

Table 86. Pair-wise genetic distance (Jukes-Cantor) between different Gallus species and Gallus gallus subspecies for 448 nucleotide positions in 16S rRNA gene

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]
[1]	-																
[2]	0.003	-															
[3]	0.003	0.000	-														
[4]	0.003	0.000	0.000	-													
[5]	0.003	0.000	0.000	0.000	-												
[6]	0.003	0.000	0.000	0.000	0.000	-											
[7]	0.003	0.000	0.000	0.000	0.000	0.000	-										
[8]	0.003	0.000	0.000	0.000	0.000	0.000	0.000	-									
[9]	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-								
[10]	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-							
[11]	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-						
[12]	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-					
[13]	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-				
[14]	0.015	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	-			
[15]	0.029	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.017	-		
[16]	0.017	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.008	0.022	-	
[17]	0.066	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.058	0.064	0.062	-

Note : **[1]**-G.g. murghi; **[2]**-G.g. domesticus (WLH); **[3]**-G.g. domesticus (WLH); **[4]** G.g. domesticus (WLH); **[5]** G.g.domesticus (WPR); **[6]** G.g.domesticus (NL); **[7]** G.g.domesticus (NH); **[8]**G.g. domesticus (T); **[9]** G.g.domesticus (SL); **[10]** G.g.gallus1; **[11]** G.g.gallus2; **[12]** G.g.bankiva; **[13]** G.g.spadicus; **[14]** G.lafayettei; **[15]** G.sonneratii; **[16]** G.varius; **[17]** N.meleagris

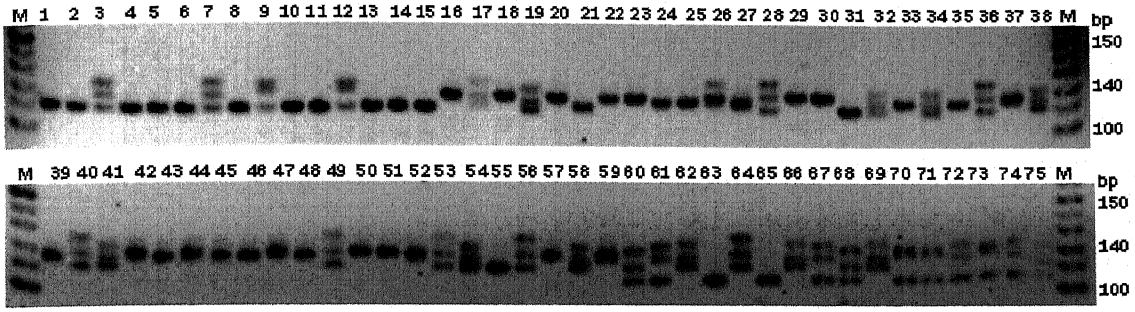


Fig 1. Microsatellite allelic profile with ADL 268. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 10 bp ladder, Bangalore Genei

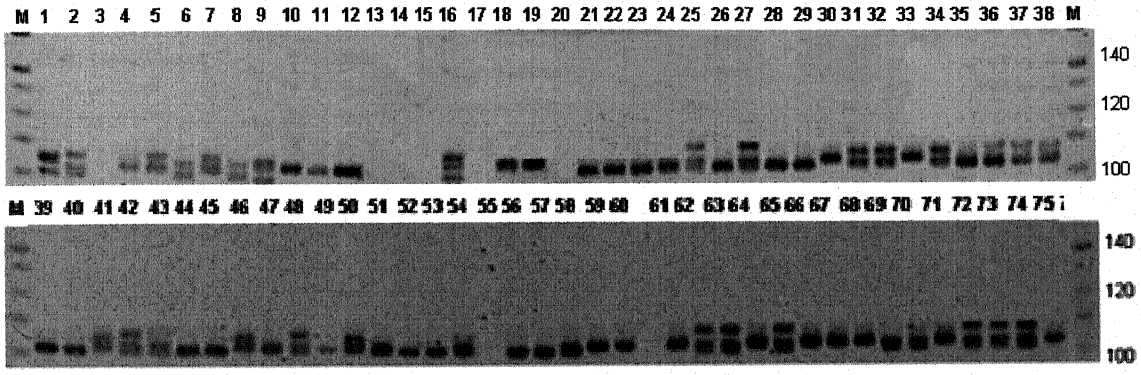


Fig 2. Microsatellite allelic profile with MCW 111. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 25 bp ladder, Invitrogen

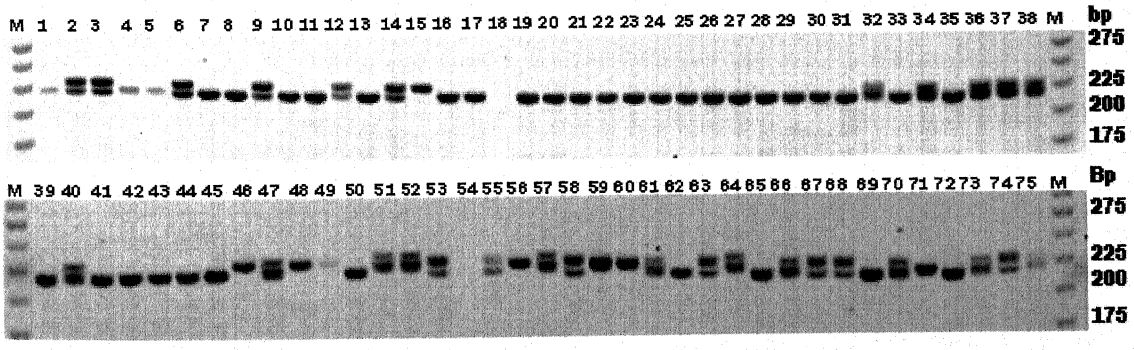


Fig 3. Microsatellite allelic profile with MCW 248. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 25 bp ladder, Invitrogen

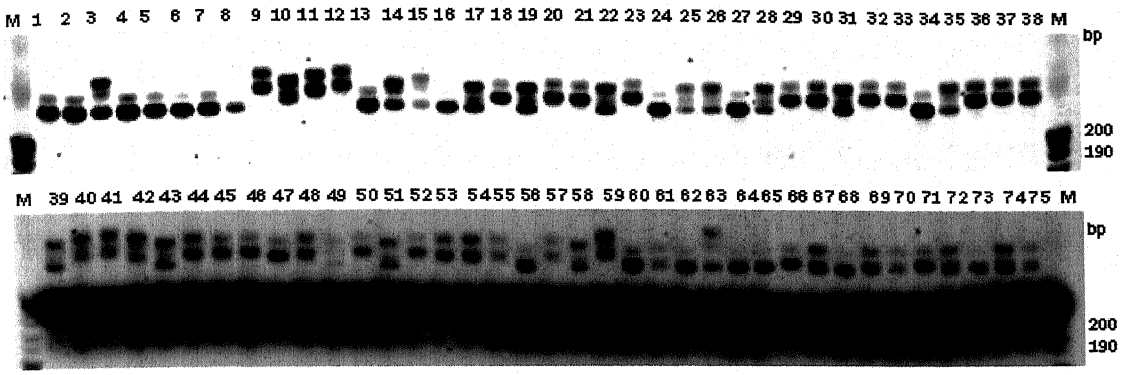


Fig 4. Microsatellite allelic profile with MCW 34. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 10 bp ladder, Bangalore Genei

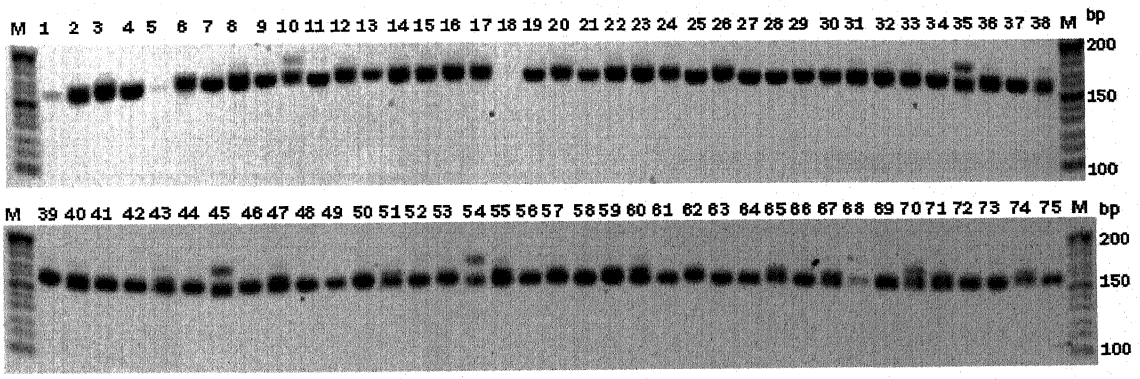


Fig 5 . Microsatellite allelic profile with MCW 37. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 10 bp ladder, Banlore Genei

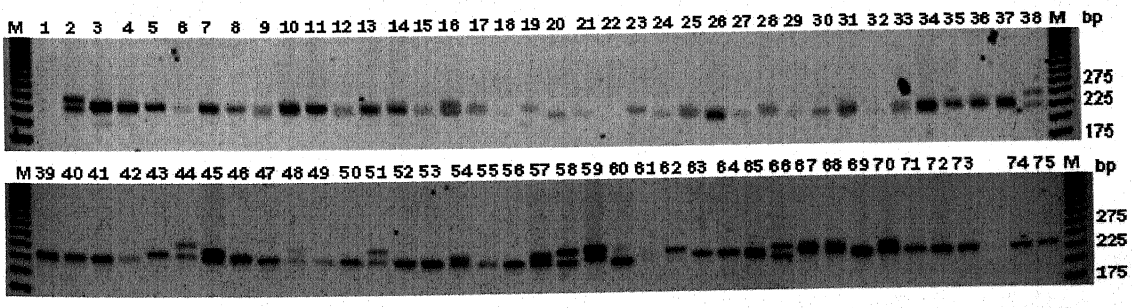


Fig 6 . Microsatellite allelic profile with MCW 206. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 25 bp ladder, Invitrogen

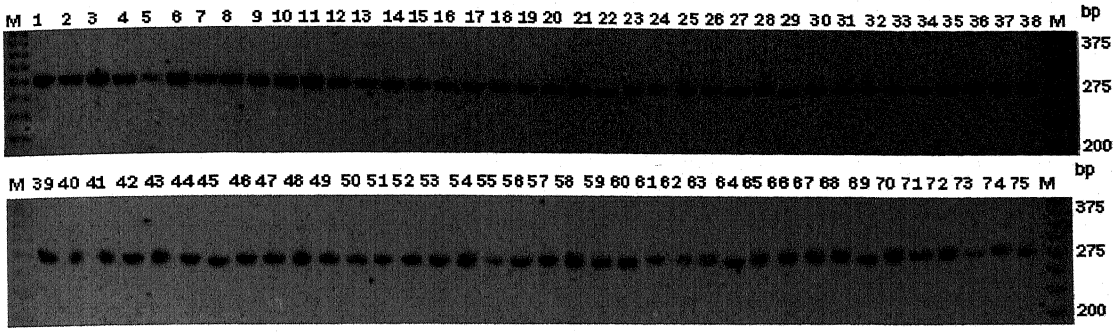


Fig 7. Microsatellite allelic profile with MCW 103. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 10 bp ladder, Bangalore Genei

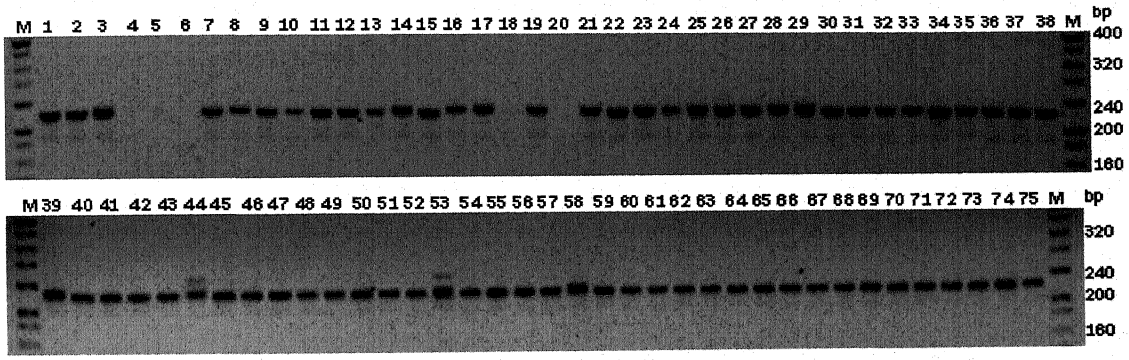


Fig 8. Microsatellite allelic profile with MCW 222. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 20 bp ladder, Banlore Genei

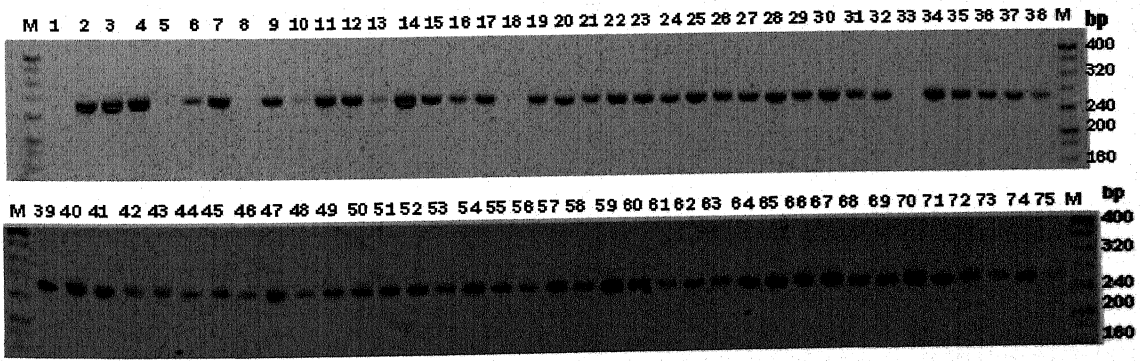


Fig 9. Microsatellite allelic profile with MCW 98. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 20 bp ladder, Banlore Genei

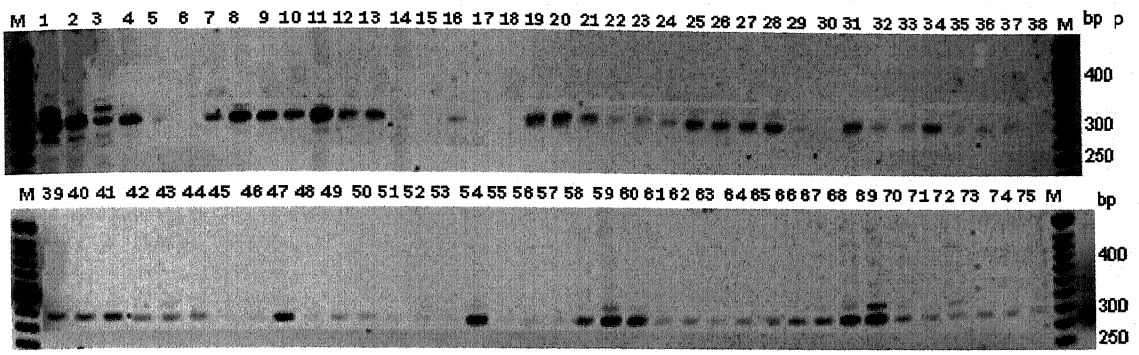


Fig 10 . Microsatellite allelic profile with MCW 284. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 25 bp ladder, Invitrogen

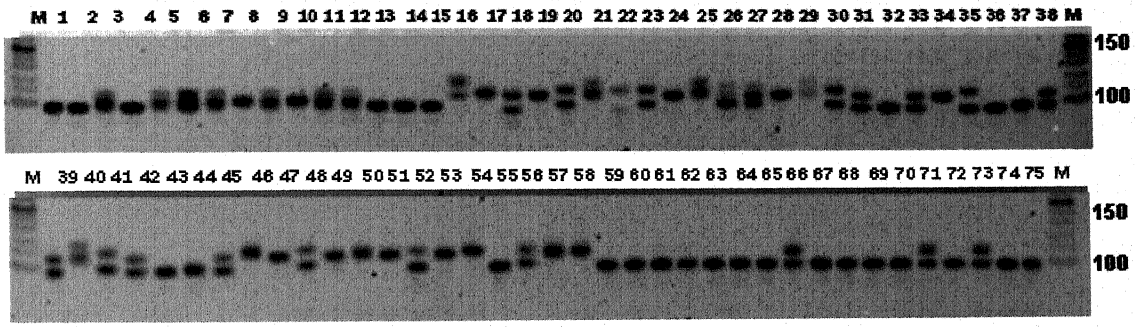


Fig 11. Microsatellite allelic profile with MCW 295. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 10 bp ladder, Bangalore Genei

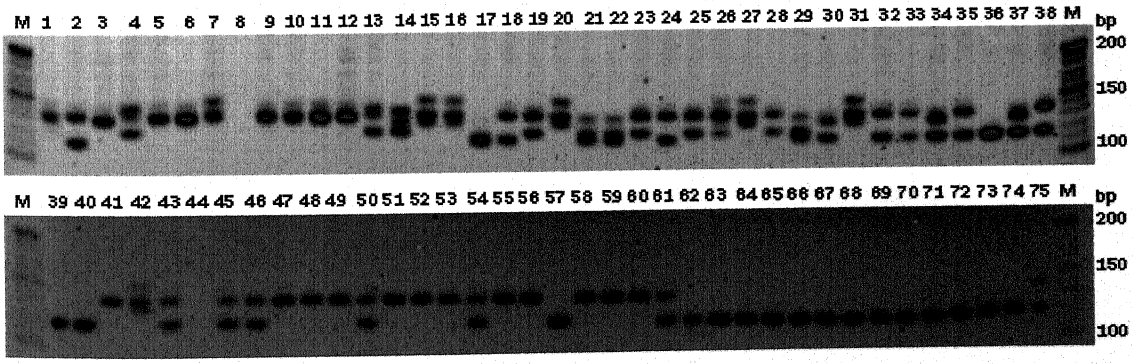


Fig 12. Microsatellite allelic profile with MCW 81. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 10 bp ladder, Bangalore Genei

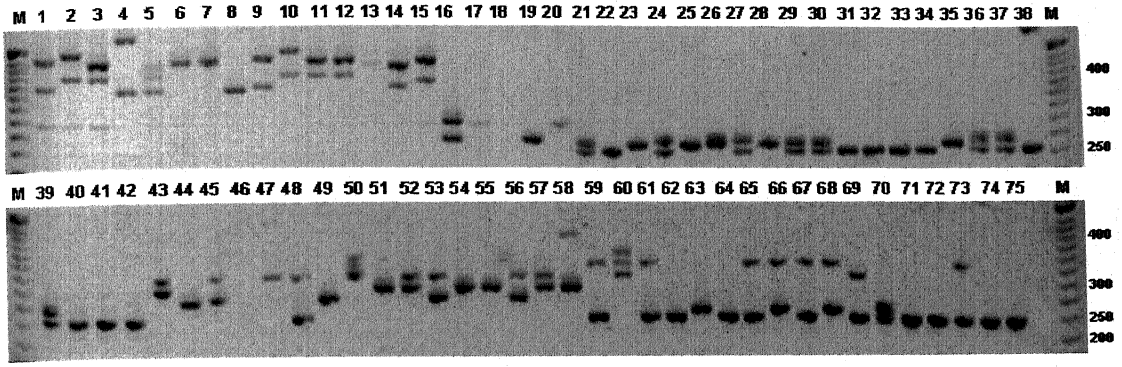


Fig 13. Microsatellite allelic profile with LEI 192. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 20 bp ladder, Bangalore Genei

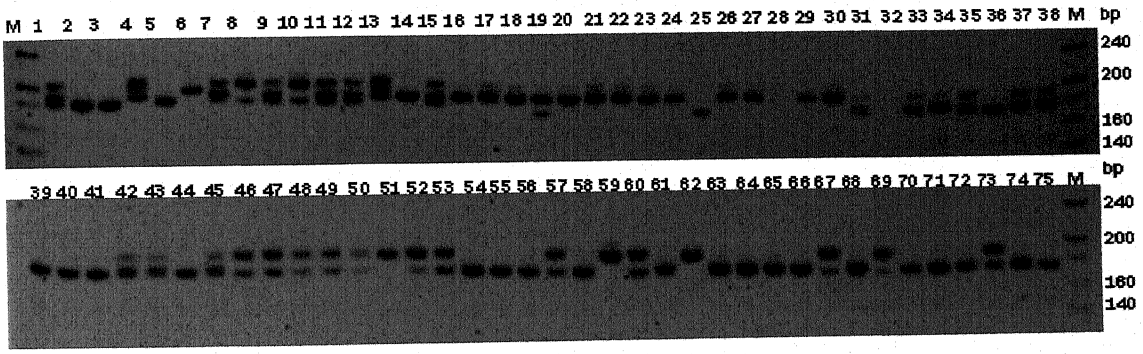


Fig 14. Microsatellite allelic profile with MCW 14. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 20 bp ladder, Bangalore Genei

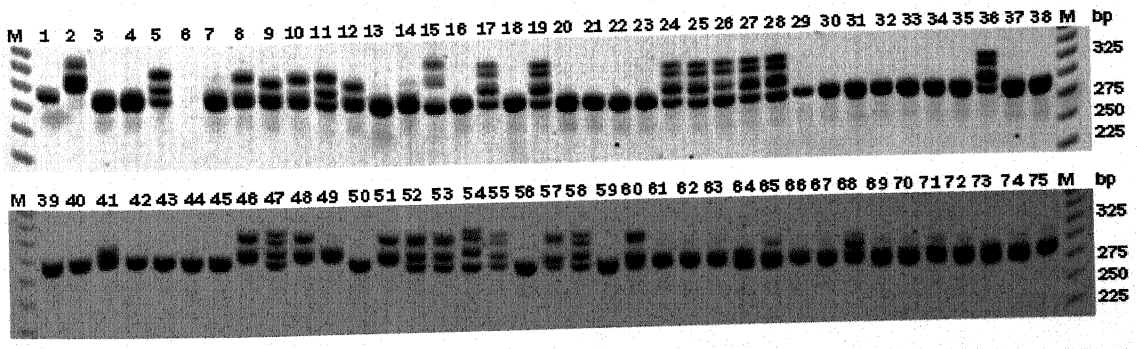


Fig 15. Microsatellite allelic profile with MCW 183. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 25 bp ladder, Invitrogen

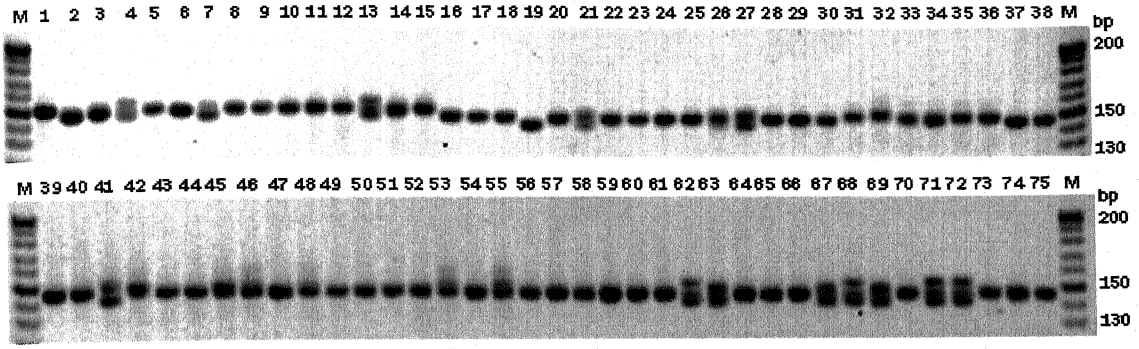


Fig 16. Microsatellite allelic profile with MCW 78. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 10 bp ladder, Bangalore Genei

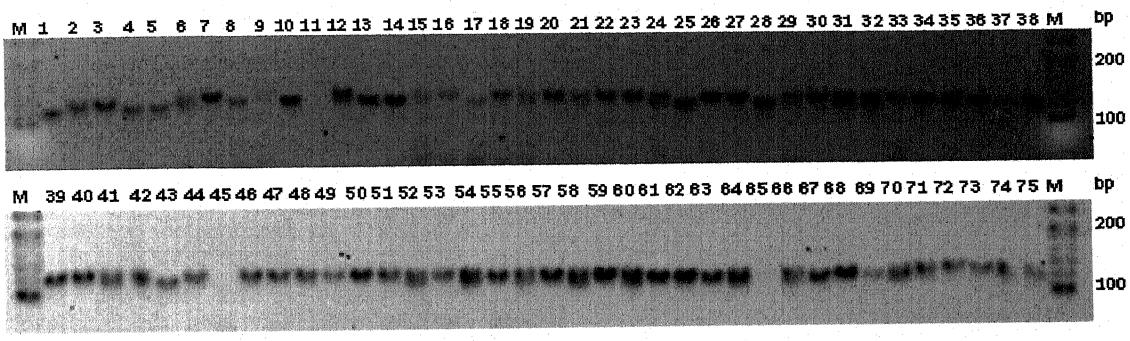


Fig 17. Microsatellite allelic profile with ADL 278. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 20 bp ladder, Bangalore Genei

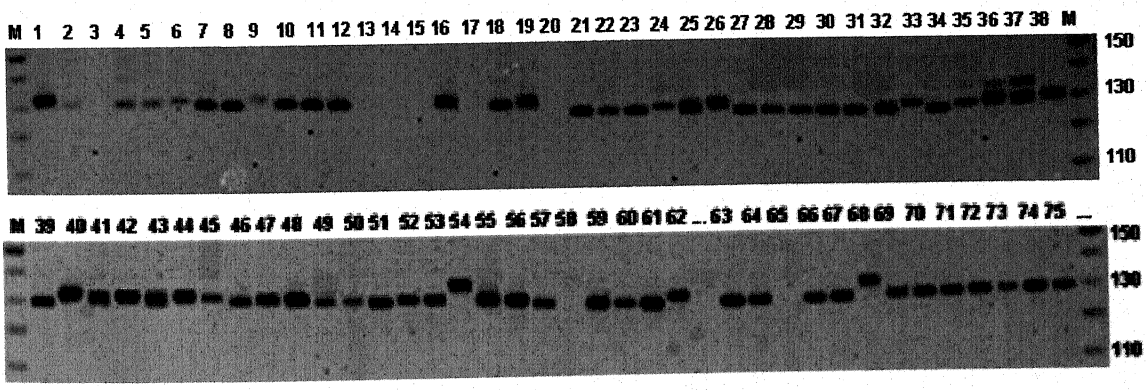


Fig 18. Microsatellite allelic profile with ADL 112. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 25 bp ladder, Invitrogen

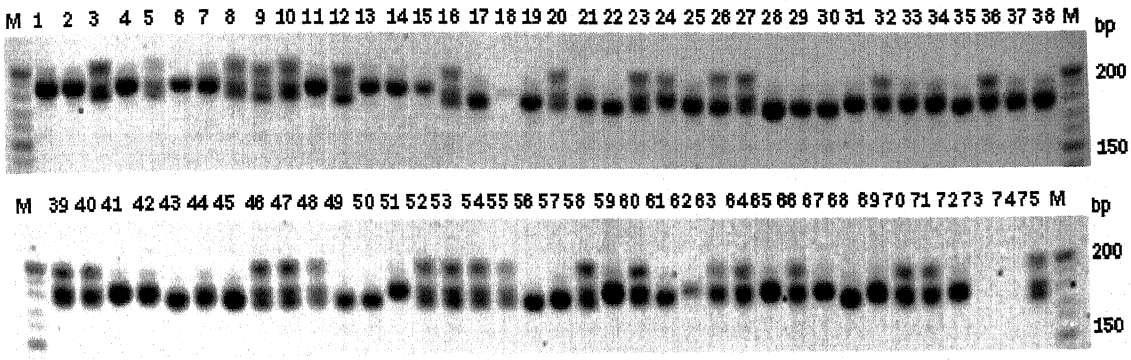


Fig 19. Microsatellite allelic profile with MCW 67. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 10 bp ladder, Bangalore Genei

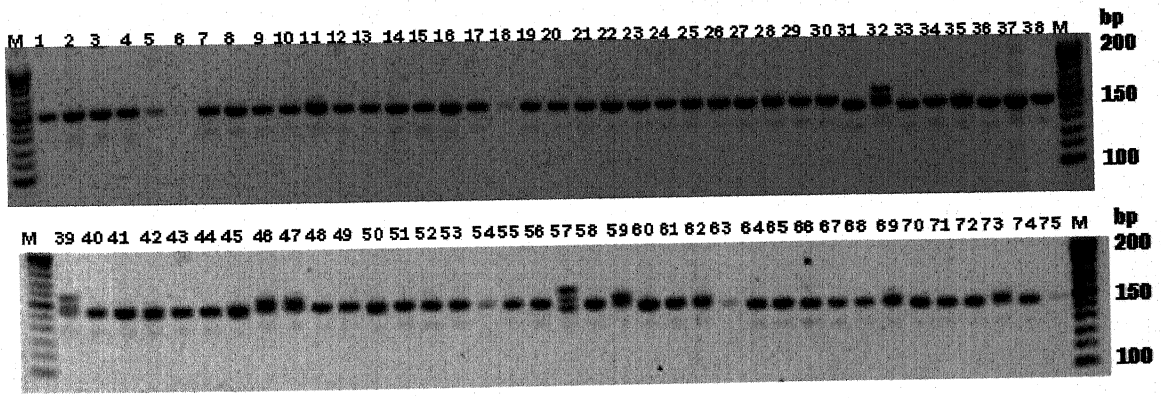


Fig 20. Microsatellite allelic profile with MCW 216. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 10 bp ladder, Banlore Genei

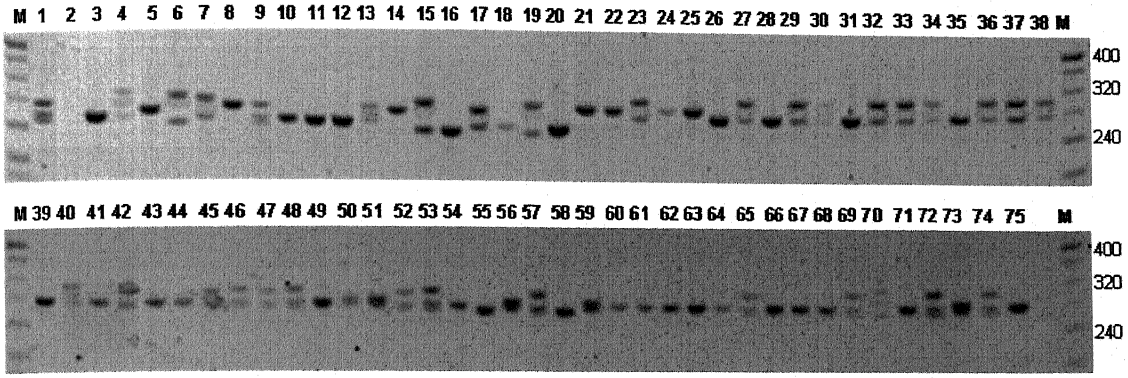


Fig 21. Microsatellite allelic profile with MCW 330. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 10 bp ladder, Banlore Genei

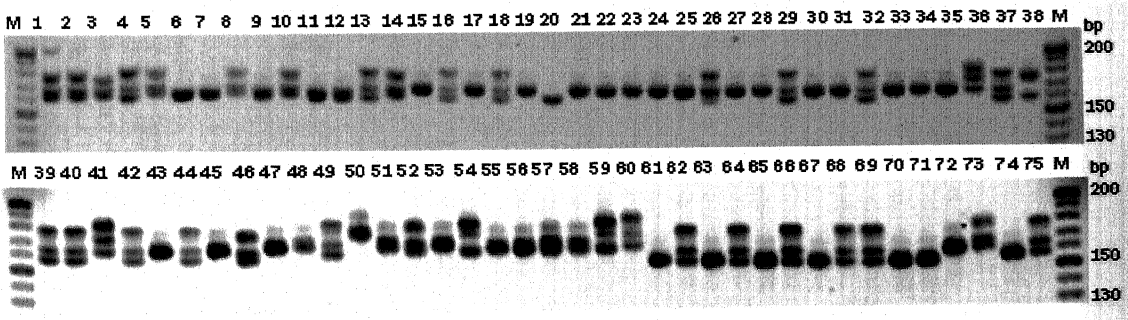


Fig 22 . Microsatellite allelic profile with MCW 69. Lane 1-15 : RJF, 16-30 : WL, 31-45 : KN, 46-60 : AS, 61-75 : RC. M : 10 bp ladder, Bangalore Genei

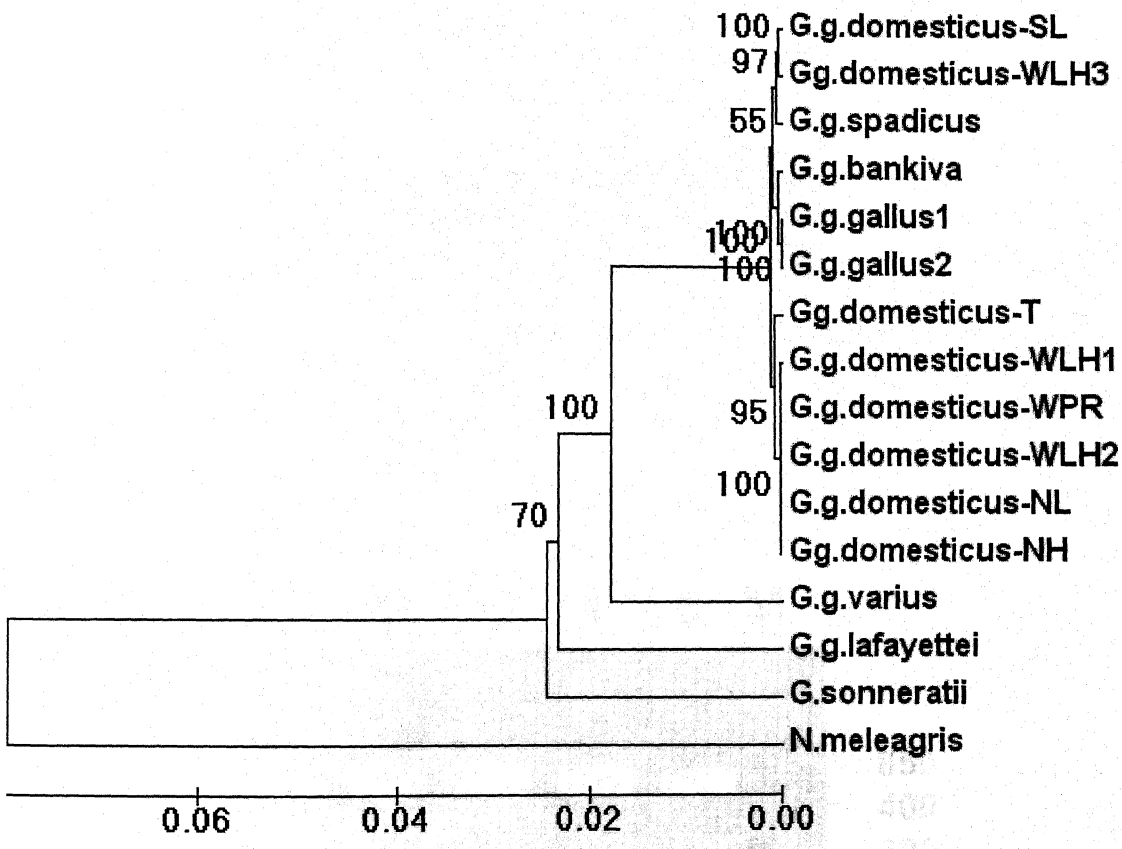


Fig 23. Phylogenetic tree based on nucleotide variation over 14037 nucleotides for different mitochondrial genes. The phylogenetic tree was outgrouped-rooted by the guinea fowl (*Numida meleagris*). Values at nodes represent bootstrap replication sscores (based on 100 resamplings).

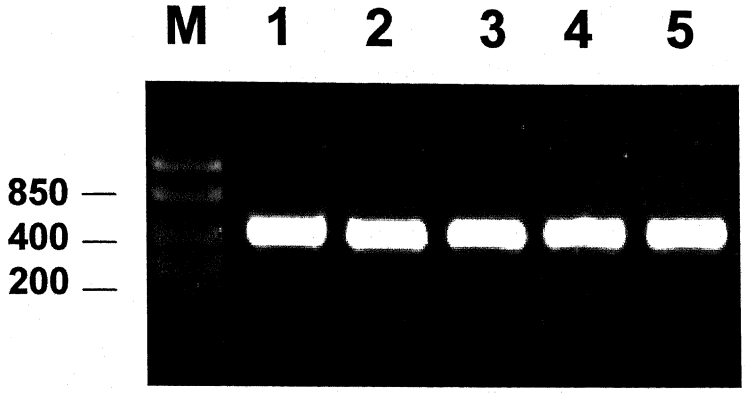


Fig 24. Polymerase Chain Reaction (PCR) amplification of partial 12S rRNA gene. 1 : RJF, 2 : WL, 3 : KN, 4 : AS, 5 : RC. M : molecular size marker (Low range Fast ruler, Fermentas)

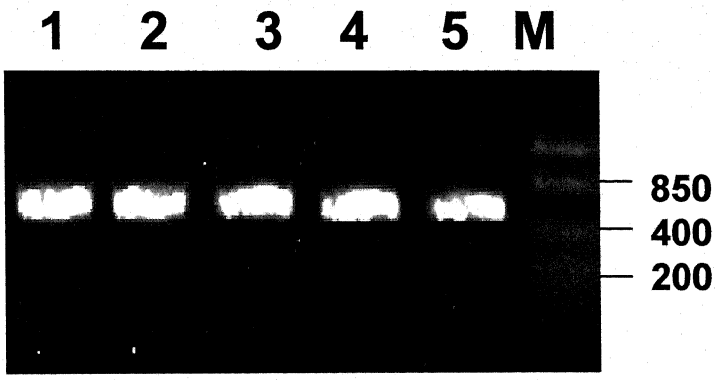
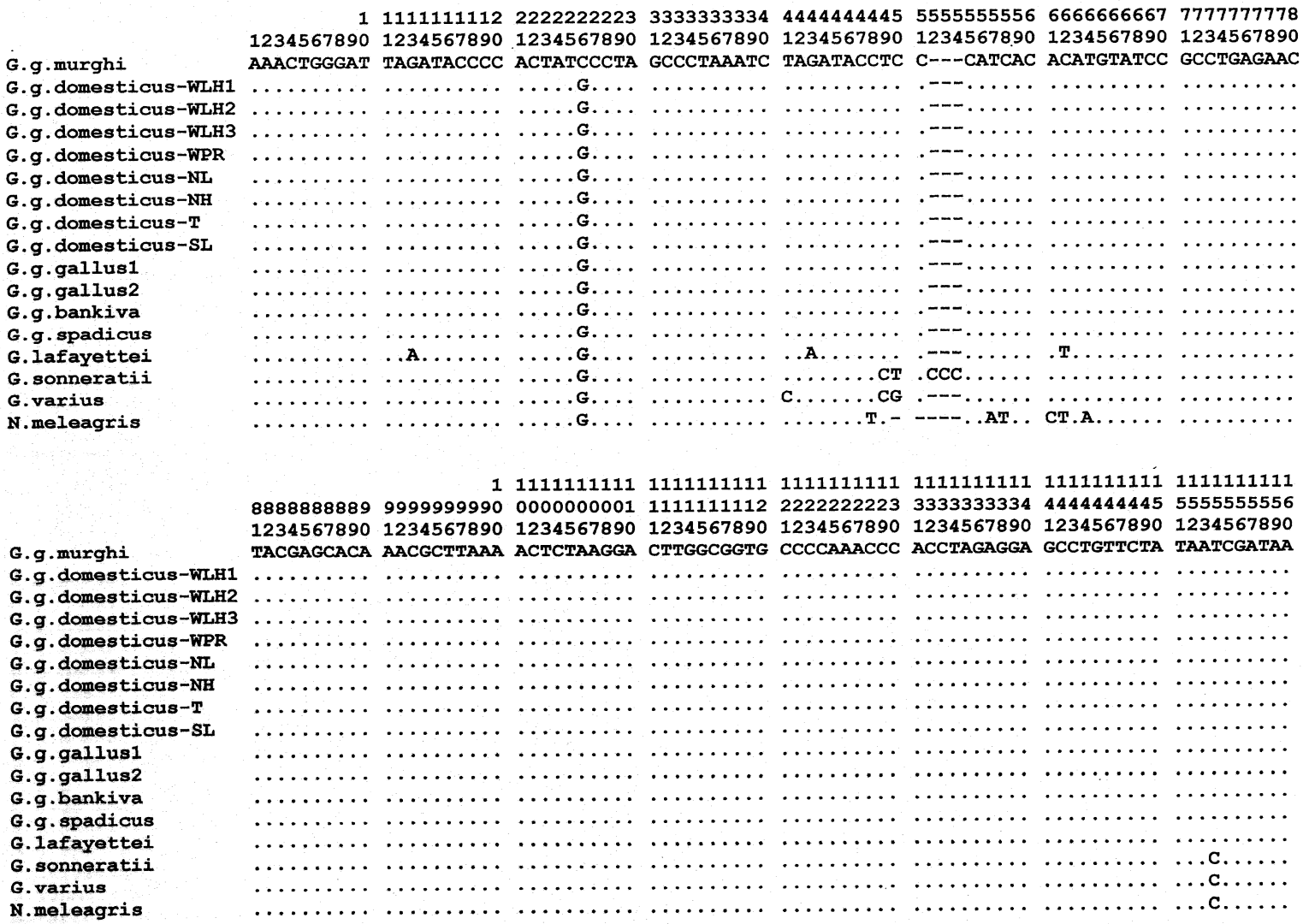


Fig 25. Polymerase Chain Reaction (PCR) amplification of partial 16S rRNA gene. 1 : RJF, 2 : WL, 3 : KN, 4 : AS, 5 : RC. M : molecular size marker (Low range Fast ruler, Fermentas)


1 1111111112 2222222223 3333333334 4444444445 5555555556 6666666667 7777777778
1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
G.g.murghi AAACTGGGAT TAGATACCCC ACTATCCCTA GCCCTAAATC TAGATACCTC C---CATCAC ACATGTATCC GCCTGAGAAC
G.g.domesticus-WLH1 ....G.... ....---..... ....
G.g.domesticus-WLH2 ....G.... ....---..... ....
G.g.domesticus-WLH3 ....G.... ....---..... ....
G.g.domesticus-WPR ....G.... ....---..... ....
G.g.domesticus-NL ....G.... ....---..... ....
G.g.domesticus-NH ....G.... ....---..... ....
G.g.domesticus-T ....G.... ....---..... ....
G.g.domesticus-SL ....G.... ....---..... ....
G.g.gallus1 ....G.... ....---..... ....
G.g.gallus2 ....G.... ....---..... ....
G.g.bankiva ....G.... ....---..... ....
G.g.spadicus ....G.... ....---..... ....
G.lafayettei ....A..... .....G.... ....A..... .---..... .T..... .....
G.sonneratii ....G.... ....CT .CCC..... ....
G.varius ....G.... ..... C.....CG .---..... ....
N.meleagris ....G.... ....T.- ----..AT.. CT.A..... .....
1 1111111111 1111111111 1111111111 1111111111 1111111111 1111111111
8888888889 9999999990 0000000001 1111111112 2222222223 3333333334 4444444445 5555555556
1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
G.g.murghi TACGAGCACA AACGCTTAAA ACTCTAAGGA CTTGGCGGTG CCCCAAACCC ACCTAGAGGA GCCTGTTCTA TAATCGATAA
G.g.domesticus-WLH1 ....
G.g.domesticus-WLH2 ....
G.g.domesticus-WLH3 ....
G.g.domesticus-WPR ....
G.g.domesticus-NL ....
G.g.domesticus-NH ....
G.g.domesticus-T ....
G.g.domesticus-SL ....
G.g.gallus1 ....
G.g.gallus2 ....
G.g.bankiva ....
G.g.spadicus ....
G.lafayettei ....
G.sonneratii ....C.....
G.varius ....C.....
N.meleagris ....C.....

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                        1111111111 1111111111 1111111111 1111111112 2222222222 2222222222 2222222222 2222222222
                        6666666667 7777777778 8888888889 9999999990 0000000001 1111111112 2222222223 3333333334
                        1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
G.g.murghi              TCCACGATTC ACCCAACCAC CCCTTGCCAG CACAGCCTAC ATACCGCCGT CGCCAGCCCA CCTCTAATGA AAGAACAACA
G.g.domesticus-WLH1     .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH2     .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH3     .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WPR      .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NL       .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NH       .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-T        .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-SL       .......... .......... .......... .......... .......... .......... .......... ..........
G.g.gallus1             .......... .......... .......... .......... .......... .......... .......... ..........
G.g.gallus2             .......... .......... .......... .......... .......... .......... .......... ..........
G.g.bankiva             .......... .......... .......... .......... .......... .......... .......... ..........
G.g.spadicus            .......... .......... .......... .......... .......... .......... .......... ..........
G.lafayettei            ........C. ....G..... .......... .......... .......... .......... ....C..... ..........
G.sonneratii            ........C. .......... .......... .......... .......... .......... ....C..... ..........
G.varius                ........C. .......... .........A .......... .......... .......... .......... ..........
N.meleagris             ........C. .......... .T.......A .......... .......... .......... ...AC.-... ...CG.....

                        2222222222 2222222222 2222222222 2222222222 2222222222 2222222223 3333333333 3333333333
                        4444444445 5555555556 6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
                        1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
G.g.murghi              GTGAGCTCAA TAGCCCCTC- -GCTAATAAG ACAGGTCAAG GTATAGCCTA TGG-GGTGGG AGAAATGGGC TACATTTTCT
G.g.domesticus-WLH1     .......... .........- -......... .......... .......... ...-.....A .......... ..........
G.g.domesticus-WLH2     .......... .........- -......... .......... .......... ...-...... .......... ..........
G.g.domesticus-WLH3     .......... .........- -......... .......... .......... ...-...... .......... ..........
G.g.domesticus-WPR      .......... .........- -......... .......... .......... ...-...... .......... ..........
G.g.domesticus-NL       .......... .........- -......... .......... .......... ...-...... .......... ..........
G.g.domesticus-NH       .......... .........- -......... .......... .......... ...-A..... .......... ..........
G.g.domesticus-T        .......... .........- -......... .......... .......... ...-...... .......... ..........
G.g.domesticus-SL       .......... .........- -......... .......... .......... ...-...... .......... ..........
G.g.gallus1             .......... .........- -......... .......... .......... ...-...... .......... ..........
G.g.gallus2             .......... .........- -......... .......... .......... ...-...... .......... ..........
G.g.bankiva             .......... .........- -......... .......... .......... ...-...... .......... ..........
G.g.spadicus            .......... .........- -......... .......... .......... ...-...... .......... ..........
G.lafayettei            .......... .......C.C C....G.... .......... .......... ...-.....A .......... ..........
G.sonneratii            .......... .......C.- -.....C... .......... .......... ...-.....A .......... ..........
G.varius                .......... ......TC.- -......... .......... .......T.. ...-.....A .......... ..........
N.meleagris             .......... C..T...CA- --....C... .......... ........C. ...A.....A .......... ..........
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                        3333333333 3333333333 3333333333 3333333333 3333333333 3333333333 3333333333 3333333334
                        2222222223 3333333334 4444444445 5555555556 6666666667 7777777778 8888888889 9999999990
                        1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
G.g.murghi              AACATAGAAC AA--ACGAAA AAGGACGTGA AACCCGCCCT TAGAAGGAGG ATTTACGCAG TAAAGTGAGA TCATACCCCC
G.g.domesticus-WLH1     .......... ..--...... .......... .......... .......... .....-.... .......... ..........
G.g.domesticus-WLH2     .......... ..--...... .......... .......... .......... .....-.... .......... ..........
G.g.domesticus-WLH3     .......... ..--...... .....T.... .......... .......... .....-.... .......... ..........
G.g.domesticus-WPR      .......... ..--...... .......... .......... .......... .....-.... .......... ..........
G.g.domesticus-NL       .......... ..--...... .......... .......... .......... .....-.... .......... ..........
G.g.domesticus-NH       .......... ..--...... .......... .......... .......... .....-.... .......... ..........
G.g.domesticus-T        .......... ..--...... .......... .......... .......... .....-.... .......... ..........
G.g.domesticus-SL       .......... ..--...... .....T.... .......... .......... .....-.... .......... ..........
G.g.gallus1             .......... ..--...... .......... .......... .......... .....-.... .......... ..........
G.g.gallus2             .......... ..--...... .......... .......... .......... .....-.... .......... ..........
G.g.bankiva             .......... ..--...... .......... .......... .......... .....-.... .......... ..........
G.g.spadicus            .......... ..--...... .......... .......... .......... .....-.... .......... ..........
G.lafayettei            .G........ .G--...... .......... .......... .G........ .....-.... .......... C....TA...
G.sonneratii            .......... ..--...... .......... .......... .G........ .....-.... ......A... C......T..
G.varius                .G........ ..--...... .......... .......... .G........ .....-.... .......... C......-..
N.meleagris             .G........ .CTC...... ...AG.A... ....T..... .......... .....-.... ....A..G.. C.....AT.T

                        4444444444 4444444444 4444444444 4444444444 4444444444 44
                        0000000001 1111111112 2222222223 3333333334 4444444445 55
                        1234567890 1234567890 1234567890 1234567890 1234567890 12
G.g.murghi              TAAGCTCACT TTAAGACGGC TCTGAGGCAC GTACACACCG CCCGTCACCC TC
G.g.domesticus-WLH1     .......... .......... .......... .....T.... .......... ..
G.g.domesticus-WLH2     .......... .......... .......... .....T.... .......... ..
G.g.domesticus-WLH3     .......... .......... .......... .....T.... .......... ..
G.g.domesticus-WPR      .......... .......... .......... .....T.... .......... ..
G.g.domesticus-NL       .......... .......... .......... .....T.... .......... ..
G.g.domesticus-NH       .......... .......... .......... .....T.... .......... ..
G.g.domesticus-T        .......... .......... .......... .....T.... .......... ..
G.g.domesticus-SL       .......... .......... .......... .....T.... .......... ..
G.g.gallus1             .......... .......... .......... .....T.... .......... ..
G.g.gallus2             .......... .......... .......... .....T.... .......... ..
G.g.bankiva             .......... .......... .......... .....T.... .......... ..
G.g.spadicus            .......... .......... .......... .....T.... .......... ..
G.lafayettei            .....C.... .......... .......... .....T.... .......... ..
G.sonneratii            .G....T... .......... C......... .....T.... .......... ..
G.varius                .......... ......T... .......... .....T.... .......... ..
N.meleagris             .....C..T. .....C.... ....G..... .....T.... .......... ..
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Fig 26. Multiple sequence alignment of partial 12S rRNA fragment from G. g. murghi with corresponding sequences from other chicken breeds, gallus gallus subspecies and gallus species.

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                                1 1111111112 2222222223 3333333334 4444444445 5555555556 6666666667 7777777778
                       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
G.g.murghi             CGCCTGTTTA CCAAAAACAT AGCCTTCAGC TAACAACAAG TATTGAAGGT GATGCCTGCC CAGTGACCCC CAAAGTTCAA
G.g.domesticus-WLH1    ..A......C .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH2    ..A......C .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH3    ..A......C .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WPR     ..A......C .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NL      ..A......C .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NH      ..A......C .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-T       ..A......C .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-SL      ..A......C .......... .......... .......... .......... .......... .......... ..........
G.g.gallus1            ..A......C .......... .......... .......... .......... .......... .......... ..........
G.g.gallus2            ..A......C .......... .......... .......... .......... .......... .......... ..........
G.g.bankiva            ..A......C .......... .......... .......... .......... .......... .......... ..........
G.g.spadicus           ..A......C .......... .......... .......... .......... .......... .......... ..........
G.lafayettei           ..A......C .......... .......... C.G....... .......... .......... .......... T.........
G.sonneratii           ..A......C .......... .......... C..T...... .......... .......... .......... ..........
G.varius               ..A......C .......... .......... .......... .......... .......... .......... ..........
N.meleagris            ..A......C .......... .......... A......... .......... .......... .......TA. -..C......

                                1 1111111111 1111111111 1111111111 1111111111 1111111111 1111111111
                       8888888889 9999999990 0000000001 1111111112 2222222223 3333333334 4444444445 5555555556
                       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
G.g.murghi             CGGCCGCGGT ATCCTAACCG TGCGAAGGTA GCGCAATCAA TTGTCCCGTA AATTGAGACT TGTATGAATG GCTAAACGAG
G.g.domesticus-WLH1    .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH2    .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH3    .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WPR     .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NL      .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NH      .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-T       .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-SL      .......... .......... .......... .......... .......... .......... .......... ..........
G.g.gallus1            .......... .......... .......... .......... .......... .......... .......... ..........
G.g.gallus2            .......... .......... .......... .......... .......... .......... .......... ..........
G.g.bankiva            .......... .......... .......... .......... .......... .......... .......... ..........
G.g.spadicus           .......... .......... .......... .......... .......... .......... .......... ..........
G.lafayettei           .......... .......... .......... .......... .......A.. .......... .......... ..........
G.sonneratii           .......... .......... .......... .......... .......A.. .......... .......... ..........
G.varius               .......... .......... .......... .......... .......A.. .......... .......... ..........
N.meleagris            .......... .......... .......... .......... .....T.A.. ...C...... .......... ..........
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                    1111111111 1111111111 1111111111 1111111112 2222222222 2222222222 2222222222 2222222222
                    6666666667 7777777778 8888888889 9999999990 0000000001 1111111112 2222222223 3333333334
                    1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
G.g.murghi          GTCTTAACTG TCTCCTGTAG GTAATCTATG AAATTAGTAT TCCCGTGCAA AAACGAGAAT GTGAACATAA GACGAGAAGA
G.g.domesticus-WLH1 .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH2 .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH3 .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WPR  .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NL   .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NH   .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-T    .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-SL   .......... .......... .......... .......... .......... .......... .......... ..........
G.g.gallus1         .......... .......... .......... .......... .......... .......... .......... ..........
G.g.gallus2         .......... .......... .......... .......... .......... .......... .......... ..........
G.g.bankiva         .......... .......... .......... .......... .......... .......... .......... ..........
G.g.spadicus        .......... .......... .......... .......... .......... .......... .......... ..........
G.lafayettei        .......... .......C.. .......... .......... .......... .......... ....C..... ..........
G.sonneratii        .......... .......C.. .......... .......... ..T....... .......... ....C..... ..........
G.varius            .......... .......C.. .......... .......... .......... .......... ....C..... ..........
N.meleagris         .......... ......AC.. A.G...AG.. .......... .......... .......... .G..C..... ..........

                    2222222222 2222222222 2222222222 2222222222 2222222222 2222222223 3333333333 3333333333
                    4444444445 5555555556 6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
                    1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
G.g.murghi          CCCTGTGGAA CTTTAAAATC ACGACCACCT TACAACCTTA CACAGCCCCA CTGGGTCCAC CCACACATAA ACCCC-TGGT
G.g.domesticus-WLH1 .......... .......... .......... .......... .......... .......... .......... .....-....
G.g.domesticus-WLH2 .......... .......... .......... .......... .......... .......... .......... .....-....
G.g.domesticus-WLH3 .......... .......... .......... .......... .......... .......... .......... .....-....
G.g.domesticus-WPR  .......... .......... .......... .......... .......... .......... .......... .....-....
G.g.domesticus-NL   .......... .......... .......... .......... .......... .......... .......... .....-....
G.g.domesticus-NH   .......... .......... .......... .......... .......... .......... .......... .....-....
G.g.domesticus-T    .......... .......... .......... .......... .......... .......... .......... .....-....
G.g.domesticus-SL   .......... .......... .......... .......... .......... .......... .......... .....-....
G.g.gallus1         .......... .......... .......... .......... .......... .......... .......... .....-....
G.g.gallus2         .......... .......... .......... .......... .......... .......... .......... .....-....
G.g.bankiva         .......... .......... .......... .......... .......... .......... .......... .....-....
G.g.spadicus        .......... .......... .......... .......... .......... .......... .......... .....-....
G.lafayettei        .......... .......... .......... .......... ....A..... .......... .......... .....-....
G.sonneratii        .......... .......... .........C .......CC. ....A....G .C.....T.. .......C.. .....-....
G.varius            .......... .......... .......... .......... ..T.-..... .......... ...TG..... .....C....
N.meleagris         .......... ...C...... .......... .GAC...A-. .C..AAT... .C..AC.... .......-.. ..A..-....
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                        3333333333 3333333333 3333333333 3333333333 3333333333 3333333333 3333333333 3333333334
                        2222222223 3333333334 4444444445 5555555556 6666666667 7777777778 8888888889 9999999990
                        1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
G.g.murghi              CGACATTTTT CGGTTGGGGC GACCTTGGAG AAAAAAAAAT CCTCCAAACC CACAGACCAC AACTCTTCAC TAAGACCAAC
G.g.domesticus-WLH1     .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH2     .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH3     .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WPR      .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NL       .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NH       .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-T        .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-SL       .......... .......... .......... .......... .......... .......... .......... ..........
G.g.gallus1             .......... .......... .......... .......... .......... .......... .......... ..........
G.g.gallus2             .......... .......... .......... .......... .......... .......... .......... ..........
G.g.bankiva             .......... .......... .......... .......... .......... .......... .......... ..........
G.g.spadicus            .......... .......... .......... .......... .......... .......... .......... ..........
G.lafayettei            .......... .......... .......... .......... .......... .......... .......... C.........
G.sonneratii            .......... .......... .......... .......... .......... .......... .......... C.........
G.varius                .......... .......... .......... .......... .......... .......... .......... C.........
N.meleagris             .......... .......... .......... .....T.... .......... -......... .......... CG........

                        4444444444 4444444444 4444444444 4444444444 4444444444 4444444444 4444444444 4444444444
                        0000000001 1111111112 2222222223 3333333334 4444444445 5555555556 6666666667 7777777778
                        1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
G.g.murghi              TCCTCAAAGT ACCAACAGTA ACCAGACCCA ATATAATTGA GCAATGGACC AAGCTACCCC AGGGATAACA GCGCAATCTC
G.g.domesticus-WLH1     .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH2     .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH3     .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WPR      .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NL       .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NH       .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-T        .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-SL       .......... .......... .......... .......... .......... .......... .......... ..........
G.g.gallus1             .......... .......... .......... .......... .......... .......... .......... ..........
G.g.gallus2             .......... .......... .......... .......... .......... .......... .......... ..........
G.g.bankiva             .......... .......... .......... .......... .......... .......... .......... ..........
G.g.spadicus            .......... .......... .......... .......... .......... .......... .......... ..........
G.lafayettei            .......... .......... ..T....... .......... .......... .......... .......... ..........
G.sonneratii            .......... ..T....... ..T....... .......... .......... .......... .......... ..........
G.varius                .......... .......... ..T....... .......... .......... .......... .......... ..........
N.meleagris             C....T.... ..T....... .......... .......... T........T .......... .......... ..........
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                    4444444444 4444444445 5555555555 5555555555 5555555555 5555555555 5555555555 5555555555
                    8888888889 9999999990 0000000001 1111111112 2222222223 3333333334 4444444445 5555555556
                    1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
G.g.murghi          CTCCAAGAGC CCATATCGAC AAGGAGGTTT ACGACCTCGA TGTTGGATCA GGACAACCTA ATGGTGCAAC CGCTATTAAG
G.g.domesticus-WLH1 .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH2 .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WLH3 .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-WPR  .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NL   .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-NH   .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-T    .......... .......... .......... .......... .......... .......... .......... ..........
G.g.domesticus-SL   .......... .......... .......... .......... .......... .......... .......... ..........
G.g.gallus1         .......... .......... .......... .......... .......... .......... .......... ..........
G.g.gallus2         .......... .......... .......... .......... .......... .......... .......... ..........
G.g.bankiva         .......... .......... .......... .......... .......... .......... .......... ..........
G.g.spadicus        .......... .......... .......... .......... .......... .......... .......... ..........
G.lafayettei        .......... .......... .......... .......... .......... .......... .......... ..........
G.sonneratii        .......... .......... .......... .......... .......... .......... ......T... ..........
G.varius            .......... .......... .......... .......... .......... .......... .......... ..........
N.meleagris         .......... .......... .......... .......... .......... .......... ........G. ..........

                    5555555555 5555555555 5555555555 5555555556 6666666666 6]
                    6666666667 7777777778 8888888889 9999999990 0000000001 1]
                    1234567890 1234567890 1234567890 1234567890 1234567890 1]
G.g.murghi          GGTTCGTTTG TTCAACGATT AACAGTCCTA CGTGATCTGA GTTCAGACCG G
G.g.domesticus-WLH1 .......... .......... .......... .......... .......... .
G.g.domesticus-WLH2 .......... .......... .......... .......... .......... .
G.g.domesticus-WLH3 .......... .......... .......... .......... .......... .
G.g.domesticus-WPR  .......... .......... .......... .......... .......... .
G.g.domesticus-NL   .......... .......... .......... .......... .......... .
G.g.domesticus-NH   .......... .......... .......... .......... .......... .
G.g.domesticus-T    .......... .......... .......... .......... .......... .
G.g.domesticus-SL   .......... .......... .......... .......... .......... .
G.g.gallus1         .......... .......... .......... .......... .......... .
G.g.gallus2         .......... .......... .......... .......... .......... .
G.g.bankiva         .......... .......... .......... .......... .......... .
G.g.spadicus        .......... .......... .......... .......... .......... .
G.lafayettei        .......... .......... .......... .......... .......... .
G.sonneratii        .......... .......... .......... .......... .......... .
G.varius            .......... .......... .......... .......... .......... .
N.meleagris         .......... .......... .......... .......... .......... .
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Fig 27. Multiple sequence alignment of partial 16S rRNA fragment from G. g. murghi with corresponding sequences from other chicken breeds, gallus gallus subspecies and gallus species.

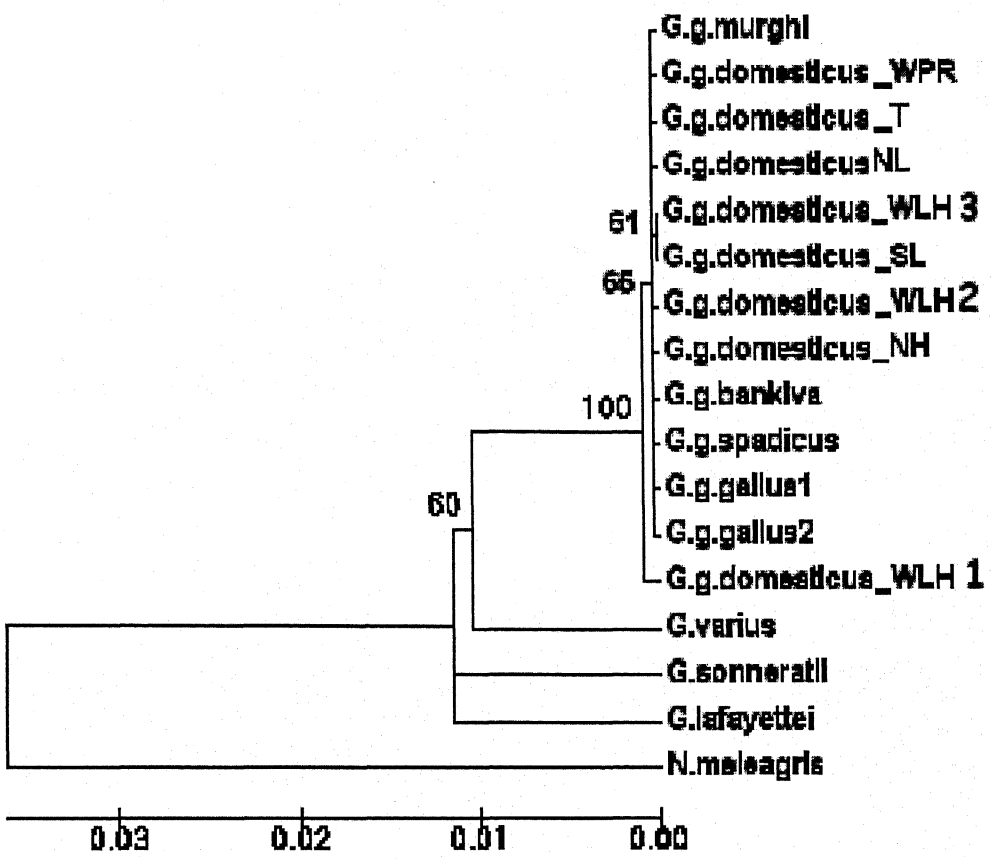

Fig 28. Phylogenetic tree based on nucleotide variation over 1063 nucleotides for partial 12S rRNA and 16S rRNA genes. The phylogenetic tree was outgrouped-rooted by the guinea fowl (*Numida meleagris*). Values at nodes represent bootstrap replication sscores (based on 100 resamplings).

Discussion

Discussion

I. Microsatellite polymorphism between red jungle fowl and domestic chicken breeds

Microsatellites consist of tandem repeats of core sequences of di, tri, or tetra nucleotide units. The basis of polymorphism for a microsatellite locus lies in the fact that differences in number of the repeat motif (core sequences) generated by unequal crossover between the repeat units during meiosis among populations/ individuals. Hence the microsatellite locus having more number of alleles due to high polymorphism are more useful in determining genetic variation and phylogenies of organism, especially when these

markers are used for studying the genetic diversity between populations of the same species (Vanhala *et al.*, 1998).

A. Microsatellite polymorphism

In present study, out of the total 22 microsatellite markers, 21 were polymorphic, while MCW 103 proved to be monomorphic in all the populations. Earlier workers also reported the very high proportion of the polymorphic markers (Vanhala *et al.* 1998, , Hillel *et al.*, 2003, Haunshi and Sharma, 2006). Hillel *et al.* (2003) found MCW 103 as polymorphic but with few alleles. Difference in the chicken populations used by Hillel *et al.* (2003) might be the reason for this discrepancy in the behaviour of this moarker. In present study, at all the 21 microsatellite loci all the populations were polymorphic except RC at MCW 222 and WL at MCW 216. Hillel et al (2003) also reported that at all the microsatellite loci, all the populations are not polymorphic. The frequency of polymorphic population ranged from 0.69 to 1.00. In our study also, the frequency of polymorphic population ranged from 0.80 to 1.00.

Across the population, total number of alleles ranged from 5 (at MCW 222 locus) to 21 (at LEI 192 locus). Average number of alleles per loci was 11.14. Hillel et al (2003) reported 4 to 23 alleles across the populations. They also reported maximum alleles at LEI 192 loci. Number of alleles as well as average number of loci reported by different workers varied widely. Crooijmans *et al.* (1996) reported average number of marker alleles was 5.8 over all lines. Similarly, Kaiser *et al.* (2000) reported the average number of alleles per primer to be 2.8 and 2.9 in two chicken populations, while Vanhala *et al.* (1998) reported 4 to 13 alleles using nine different microsatellite markers with an average of 5.7 alleles per marker among the chicken lines of different genetic origin. Cheng *et al.* (2003), using five microsatellite markers with high polymorphisms estimated reported 5 (ADL0146) to 10 alleles (ADL0136). Li *et al.* (2004), using 20 microsatellite markers in four package lines of egg-type chickens, reported 3.25 alleles / locus. Haunshi and Sharma (2006) reported 2 to 7 alleles with an average of 3.3 alleles per marker. Tomar *et al.* (2007) also

reported 3 – 6 alleles in RC, AS, RJF and WL at 5 microsatellite loci. However Romanov and Weigend (2001) compared 20 chicken populations of different origin by typing 14 microsatellites and reported 2 and 21 alleles with the mean of 11.2 alleles per locus. Similarly, Qu *et al.* (2006) reported 6 to 51 alleles in 78 indigenous chicken breeds at 27 microsatellite loci. Average number of alleles was 18.74 per locus. Muchadeyi *et al.* (2007) using 29 microsatellites and 13 indigenous populations of Zimbabwe, found 9.7 ± 5.10 alleles/locus.

This vast difference in number of alleles at different locus might be due to difference in microsatellite markers and the population by various workers. The allelic size range at various microsatellite loci were similar to as reported by Hillel *et al.* (2003) at the same loci. Earlier workers also reported the consistency in the allelic size range at specific microsatellite (Hillel *et al.*, 2003, Haunshi and Sharma, 2006).

The set of microsatellite markers used in this study was adopted from Hillel *et al.* (2003) and were the part of the Poultry Biodiversity database(http://w3.tzv.de/aviandiv/index.html). By assuming a maximum coverage of 20 cM on each side of a marker, and accounting for primers located less than 20 cM apart, the primers selected for the present study covered about 22 % of the genome. Since the microsatellite markers selected were uniformly distributed over the genome, hence represent the thorough representation of whole chicken genome. In earlier studies also, a maximal coverage of 12 to 21 % genome was made (Crooijmans *et al.*, 1996; Vanhala *et al.*, 1998, Haunshi and Sharma, 2006), while Kaiser *et al.* 2000 covered about 57 % of the genome for estimating the genetic similarity between different chicken populations.

Expected heterozygocity varied with the marker as well as between the populations. Over all the loci, average heterozygocity ranged from 0.585 in RC to 0.760 in KN, whereas across the all populations, average heterozygocity was 0.835. High heterozygocity revealed high polymorphism in

the population at these microsatellite loci. Similarly the average PIC value ranged from 0.577 in AS and RC to 0.739 in KN, whereas across the all populations, it was 0.834.

Heterozygocity is an appropriate measure of genetic variability in a population. High heterozygocity was also reported in the present study in different chicken breeds as well as Red Jungle fowl. Pandey *et al.* (2003) reported mean heterozygosity over all loci were 0.62, 0.62, and 0.61 for Nicobari, Miri, Aseel poultry, respectively. Wang DeQian *et al.* (2003) reported mean heterozygosities from 0.351 to 0.593 in Luyuan and Chahau chickens. Cheng *et al.* (2003) reported that average heterozygosity in the Shouguang chicken was the lowest (0.3327), and that in other breeds was also less than 0.40. The PIC values ranged from 0.617 (Shouguang chicken) to 0.703 (Laiwu Black chicken). Li et al. (2004) reported the heterozygosity of microsatellite markers in the range of 0.108 to 0.765 in egg type chicken. Tu *et al.* (2005) used 30 microsatellite markers to detect the genetic diversity of 8 indigenous chicken breeds in Sichuan and found that the mean heterozygosity of 8 chicken breeds was all over 0.5. The highest was the Luning chicken (0.681), and the lowest was the Jiuyuan Dark chicken. Qu *et al.* (2006) reported that the heterozygosity (H) values of the 78 chicken breeds were all more than 0.5. The average H value (0.622) and polymorphism information content (PIC, 0.573) of these breeds suggested that the Chinese indigenous chickens possessed more genetic diversity than that reported in many other countries. Tadano *et al.* (2007) evaluated the genetic diversity and relationships of 9 native Japanese long-tailed chicken breeds. While the mean expected heterozygosity ranged from 0.293 (Koeyoshi) to 0.545 (Satsumadori), mean polymorphic information content ranged 0.250 (Koeyoshi) to 0.478 (Satsumadori), respectively. Shahbazi (2007) reported lowest heterozygosity Isfahan population (62%) and the greatest in the populations from West Azerbaijan and Mazandaran (79%). These results clearly revealed that all the markers used were moderate to highly polymorphic, hence were capable of

detecting the underlying genetic variability within as well as between the populations.

B. Population specific alleles

A total of 234 alleles were score across the 5 populations and 21 microsatellite loci and 186 alleles were found in more than 1 population. For the five populations, 48 population specific alleles were identified, and majority of them i.e. 33 alleles were present at the frequency of 0.10 and more. Out of these 48 population specific alleles, 21 were specific to RJF, 9 were specific to WL, 6 were specific to KN, 10 were specific to AS and 2 were specific to RC. Among the 21 RJF specific alleles, 6 alleles at LEI 192 locus and 4 alleles at MCW 14 locus were more important from the RJF identification point of view as these alleles, are on extreme side of the allelic range. Considering all the chicken population together, there are 114 alleles, which are present in domesticated chicken, but absent in RJF. Hillel *et al.* (2003), using the same microsatellite markers, score 213 different alleles were the 52 populations and the 22 marker loci. Most of these alleles (181) were found in more than one population. For the 52 populations, the 32 private alleles were identified. RJF had eight private alleles which were absent in the domesticated gene Pool, while taken together, the 50 domesticated populations, 91 alleles were missing in the two RJF populations. Zhou and Lamont (1999) reported line-specific alleles among breeds and lines i.e. Leghorn, Jungle Fowl, Fayoumi and Spanish breeds. Wardecka *et al.* (2004) determined microsatellite polymorphism in Rhode Island Red (RIR) and Sussex (SX) chickens, divergently selected over six generations for high (H) or low (L) incidence of skeletal defects in embryos (30.7% for H lines, 3.7% for L lines). The polymorphism analysis covered 15 microsatellite markers within four lines (a total of 60 individuals). Eight alleles were identified as specific to H lines and six alleles as specific to L lines. Nakamura *et al.* (2006) used 25 microsatellite markers to identify the polymorphism between 4 strains of Nagoya breed (native to Japan) from other breeds and commercial stocks of chicken. In these strains, 5 of the markers (ABR0015, ABR0257, ABR0417,

ABR0495, and ADL0262) had a single allele, while no other chicken breeds and hybrids had the same allele combination as the Nagoya breed strains. Hence, these 5 microsatellite markers provide a practical method to accurately discriminate the Nagoya breed from other chicken breeds. Rikimaru and Takahashi (2007) successfully discriminated the Hinai-jidori chicken from other chickens on the basis of Hinai-jidori specific alleles at 14 marker loci i.e. ABR1003, ADL0250, ABR0241, ABR0311, ABR1004, ABR1013, ABR0633, ABR1005, ABR0089, ABR1007, ABR1001, ABR1009, ABR1010, and ABR1011. Hence the microsatellite assay can effectively be used in discriminating a breed / line from other populations by identified population specific alleles.

C. Within as well as Between Population Genetic Similarity

Within population genetic similarity (WF) in different populations was estimated in terms of the band frequency. Based on the band frequency across all the microsatellite loci, low within population genetic similarity was observed in all the population as the WF estimates were 0.289, 0.314, 0.217, 0.324 and 0.360 in RJF, WL, KN, AS and RC populations, respectively. These low levels of genetic similarity revealed that the population was quite variable at these microsatellite loci. With the same microsatellite markers, Hillel et al (2003) also reported moderate to low genetic similarity within the population in most of the populations. The average Nei estimates of genetic similarity within population was 0.44. Tomar *et al.* (2007) using 5 microsatellite markers, estimated comparatively higher within-breed genetic similarity i.e. 0.646, 0.659, 0.759 and 0.693 in AS, RC, WL and RJF populations respectively, based on band sharing proportions.

Similarly, based on the band frequency across the all microsatellite loci, the genetic similarity estimates between the population ranged from 0.181 to 0.312. RJF showed low genetic similarity with the chicken breeds. At this low level of genetic similarity, WL seemed to be more close to RJF and RC

seemed to most distant. Among the different chicken breeds, WL showed very low genetic similarity with AS and RC (0.182 – 0.185) in comparison to KN (0.312). Similarly RC showed more genetic similarity with KN and AS (0.256 – 0.298) in comparison to RJF and KN (0.181 – 0.185). Both the native breeds showed more genetic similarity with each other (0.303) in comparison to other breeds. Similarly, pooled over the markers, the estimates of genetic distances between the populations ranged from 1.193 to 1.705 and reflected more or less similar trend.

Romanov and Weigend (2001) studied the phylogenic relationship between red jungle fowl and other chicken breeds and found three major phylogenic groupings were formed. One involving the Red Jungle Fowl (*Gallus gallus*), second comprising the commercial layer lines and chicken breeds that were subjected to intensive selection and the third group encompassed the German native breed populations.

Kaiser *et al.* (2001) also reported lower heterozygosity in a closed flock as compared to the open flocks using microsatellite markers, which are expected to have higher genetic variability within themselves as compared to closed flocks.

Vanhala *et al.* (1998) constructed a consensus phylogenetic tree using Neighbor-joining method, in which they observed that similar type of populations cluster together, while the diverse populations make different cluster. They reported three different clusters, one for WLH lines, second for two landrace breeds and third for RIR and broiler lines. Similarly, Zhou and Lamont (1999) also reported the high BS values (0.74 to 0.96) between line pairs with the same genetic background, while the BS estimates were low between the White Leghorn lines from different genetic bases.

Kaiser *et al.* (2000), using microsatellite markers also reported high genetic similarity between the closely related populations as compared to diverse populations. Romanov and Weigend (2001) constructed a phylogenetic tree between domestic and jungle fowl populations using Nei

standard distance and Neighbor joining method. They observed three major phylogenetic tree groupings. While the red jungle fowl formed one branch, commercial layer lines and selected chicken breeds formed the second branch; and the German native breeds formed the third one. These findings suggest the low genetic similarity between diverse lines, while the close populations had high genetic similarity between themselves. Haunshi and Sharma (2006) reported very high genetic similarity between the type A and type B BC_1 populations (0.927 ± 0.026) as both the populations were very closely related. Tomar *et al.* (2007) estimated between breed genetic similarity estimates pooled over 5 different micro satellite markers among RC, AS, WL and RJF. Estimates ranged from 0.421 between RJF and WL to 0.492 between RJF and RC. In general, RJF showed lower genetic similarity with all the other three breeds in comparison to other combinations and among three breeds, it showed maximum genetic similarity with Aseel. Similarly, the between breed genetic distances estimates pooled over different microsatellite markers ranged from 0.256 (AS and RC) to 0.856 (RJF & WL).

IIA. mt DNA polymorphism within as well as between *Gallus* species

The mitochondrial genome appears particularly suitable for establishing phylogenetic relationship among species as its high mutation rate is expected to remain constant, being relatively impervious to generation time differences between species. It may be recalled that an organism does not start its life with a single copy but with hundreds of thousands of copies of the mitochondrial genome harbored by egg cytoplasm. Accordingly, generation changes do not constitute significant epochs in the life history of mitochondrial DNA.

Between the chicken breeds very low nucleotide diversity was observed for all the mitochondrial genes. The percent sequence divergence ranged from 0.00 for ATPase8 to 0.77 for ND6. Cumulatively for all the genes, the sequence divergence was 0.33 % as over a total of 14000 nucleotides,

only 46 sites showed nucleotide substitutions. Out of these 46 nucleotide substitutions, 41 (89 %) were transitions, while 5 (11 %) were transversions. Most of these nucleotides substitutions were limited to White Leghorn(3) and Silky. These results clearly indicated the highly conserved mitochondrial DNA genes in domestic breeds. Shen *et al.* (2002) used complete cytochrome b (cyt b) gene (1140 bp) nucleotide sequences and reported high homologies observed among the chicken breeds (egg-purpose). Similarly, Wada *et al.* (2004) reported 99.77 % similarity between silkie and white leghorn as they reported 39 nucleotide differences in 16, 784 bp of complete mitochondrial DNA. Some other studies, based on nucleotide variation in hypervariable D loop region of mtDNA reported comparatively higher sequence divergence between different chicken breeds. Fu *et al.* (2001b) reported 4.45 % sequence divergence (24 polymorphic sites out of 539 bp of D –loop) among the 30 DNA sequences from native chicken breeds of Zhejiang province and the white leghorn chicken. Further, Liu *et al.* (2004) investigated the genetic variability of 544 bp mt DNA hyper variable HVSI) in a total of 48 birds belonging to 12 Chinese native chicken breeds and identified 16 from 35 polymorphic nucleotide sites, which accounted for 6.4 %.

The different red jungle fowl subspecies i.e. *G. g. spadiceus, G. g. bankiva* and *G. g. gallus* also did not show much nucleotide diversity among them. The percent sequence divergence ranged from 0.00 for ATPase8 to 0.38 for ND6, however for ND4L, it was comparatively higher (1.01 %). Cumulatively for all the genes, the sequence divergence was 0.19 % as over a total of 13996 nucleotides, only 27 sites showed nucleotide substitutions. Out of these 27 nucleotide substitutions, 22 (81 %) were transitions, while 5 (19 %) were transversions. Most of these nucleotides substitutions were limited to G. g. spadiceus. Earlier studies based nucleotide diversity in 539 bp of D loop region also revealed very low sequence divergence between G. g. gallus and G. g. spadiceus (Fu *et al.*, 2001a, Niu *et al.*, 2002), however Fumihito *et al.* (1996) observed comparatively more nucleotides diversity between *G. g.*

bankiva and other two *G. gallus* subspecies i.e. *G. g. gallus* and *G. g. spadiceus*.

The jungle fowls species showed comparatively much higher nucleotide variation between themselves. Between *G. lafayetei* and other jungle fowls, percent nucleotide divergence ranged from 1.66 to 7.27 % for different mitochondrial genes. Cumulatively over all the genes, a total of 597 nucleotides substitutions were observed, 90.28 % of which were transitions, while 9.72 % were transversions. Similarly, between *G. sonneratii* and other jungle fowls, percent nucleotide divergence ranged from 3.38 to 6.13 % for different mitochondrial genes. Cumulatively over all the genes, a total of 556 nucleotides substitutions were observed, 92.53 % of which were transitions, while 7.47 % were transversion. Between *G. varius* and other jungle fowls, percent nucleotide divergence ranged from 1.92 to 8.05 % for different mitochondrial genes. Cumulatively over all the genes, a total of 497 nucleotides substitutions were observed, 90.32 % of which were transitions, while 9.68 % were transitions. Earlier workers also reported high nucleotide divergence between different *Gallus* species (Munechika *et al.*, 1997, Fu *et al.*, 2001a, Niu *et al.*, 2002).

The domestication of chicken seems to have a long history. Earliest evidences of domestication of the chicken date back to nearly 8000 years. From 16 neolithic sites along the yellow river in Northeast China, the remains of domesticated chickens were evident and some of them dated back to 7500 years (West and Zhou, 1989). However, very strong indication of domestication of chicken was found in the Mohenjo-Doro in the Indus valley (Zeuner, 1963). The signs of domestication were also found in unlikely places like Ukraine and Spain (West and Zhou, 1989). Thus the question of single domestication site or multiple and independent domestication sites is always debatable. Earlier population studies based on isozyme polymorphism suggested the multiple and independent site of domestication (Hashiguchi *et al.*, 1983). Among the different jungle fowls, red jungle fowl (*G. gallus*) is believed to be the sole progenitor of domestic fowl. There are five subspecies

of red jungle fowl inhabiting the Indian sub-continent eastwards across Myanmar, South China, Indonesia to Java. Among these five, in India, two subspecies namely *G. g. murghi* (Indian red Jungle Fowl) and *G. g. spadicus* (Bermese red jungle fowl) are found. While the former is distributed in the north and central part of India, extending eastwards to Orissa and West Bengal, the later is confined to the Northeastern parts of India. Apart from red jungle fowl, there are three other jungle fowl species i.e. Green jungle fowl (*G. varius*), Ceylon jungle fowl (*G. lafayetei*) and Grey Jungle fowl (*G. sonerratii*). Therefore, estimation of genetic diversity between domestic fowl, red jungle fowl sub-species and other jungle fowl species and establishing phylogenetic relationship is important to have understanding of monophyletic or polyphyletic origin of domestic chicken origin.

For all the mitochondrial genes, domestic chicken (*G. g. domesticus*) consistently showed very low genetic distances with red jungle fowl subspecies (0.000 – 0.009), while with other jungle fowls, estimates were much higher (0.017-0.076). These results clearly evidenced that red jungle fowl is the sole contributor towards evolution of domestic chicken. However, regarding the genetic distances between *G. g. domesticus* and other red jungle fowl subspecies, the inconsistent trend was evident from different genes. Hence, phylogenetic analysis was made on cumulative nucleotide sequence variability for all the genes mentioned above (Fig 1). All the sub species of *G. gallus*, including *G. g. domesticus* formed single cluster, while other three jungle fowls made separate group with very high bootstrap support (71-100). Within the cluster of sub species of *G. gallus*, including *G. g. domesticus*, one sub-cluster includes exclusively *G. g. domesticus*, while other sub-cluster include *G. g. gallus*, *G. g. bankiva* and *G. g. spadicus* along with two *G. g. domesticus* breeds i.e. White leghorn and Silky with 100 % bootstrap strength. Among *G. g. bankiva*, *G. g. gallus* and *G. g. spadiceus*, former two were grouped together, while *G. g. spadiceus* was in other group. These phylogenetic analysis also clearly support very high genetic relatedness of *G. g. domesticus* with *G. gallus* however, the relative closeness of *G. g.*

domesticus with other *G. gallus* subspecies is not conclusive due to almost equal distance of all these red jungle fowl subspecies from most of the domestic chicken breeds. Present study, do not include the two *G. gallus* subspecies i.e. *G. g. murghi* (Indian red jungle fowl) and *G. g. Jaboullei* as the data on mitochondrial genome is not available on these subspecies in literature.

Earlier reports also showed the much closeness of domestic chicken (*G. g. domesticus*) with red jungle fowl in comparison to other jungle fowl species, which were clearly well distant from domestic chicken, hence exclude the possibilities of their contribution towards evolution of domestic chicken. Fu *et al.* (2001a) and Niu *et al.* (2002), based on first 539 bases of mitochondrial DNA D-loop region, reported that among the four species of the genus *Gallus*, G. g. domesticus was closest to the red jungle fowl. Similarly, Dong *et al.* (2002) reported that *G. g. domesticus* was closest the red jungle fowl (*G. gallus*) based on mt DNA D-loop polymorphism. Nishibori *et al.* (2005), based on mitochondrial D-loop analysis further supports the hypothesis developed from morphology and progeny production that red jungle fowl (RJF) is the direct ancestor of the chicken.

Regarding the differential contribution from different red jungle fowl species, based on nucleotide diversity over 14037 bp of different mitochondrial genes, the present results showed no distinct closeness of any of the three red jungle fowl sub-species i.e. *G. g. gallus*, *G. g. spadiceus* and *G. g. bankiva* could be observed as all these sub species falls in separate clusters, while majority of domestic chicken breeds made one cluster. Since two *G. gallus* subspecies were left out from this study due to lack of sequence data from these sub-species, hence the interpretation is inconclusive. However, based on RFLP and sequence of first 400 bp of control region of mitochondrial DNA, Fumihito *et al.* (1994) reported very low sequence divergence (0.5-3.0 %) between various domestic breeds of chicken and *G. g. gallus* (from Thailand), indicating a single domestication event in the area inhibited by this sub-species of the red jungle fowl. Later Fumihito *et al.* (1996)

made phylogenic analysis, on the basis of nucleotide divergence 480 bp of D loop region and observed that all domestic fowls including Indonesian races belong to same clusters with *G. g. gallus* and *G. g. spadiceus* from Thailand and its adjacent areas. However, the *G. g. gallus* from South Sumatra form a separate clusters, though the domestic chicken from same island do not belong to this cluster, but are clustered with the *G. g. gallus* and *G. g. spadiceus* from Thailand and its adjacent areas, which exclude the possibilities of an independent domestication in Sumatra from its own *G. g. gallus* and support the possibilities of transfer of Indonesian native chicken from Thailand and its adjacent area. In contrast to these reports, Liu *et al.* (2004) based on the genetic variability of 544 bp mt DNA hyper variable HVSI), reported close genetic relationship between the Chinese native chicken breeds with *G. g. gallus* as well as *G. g. spadiceus* from different areas. Similarly, phylogenetic analyses based on mtDNA hyper variable segment I (HVS-I) revealed nine highly divergent mtDNA clades (A–I) in which seven clades contained both the red jungle fowls and domestic chickens and there was no breed-specific clade in the chickens (Liu *et al.*, 2006a). Oka *et al.* (2007) analyzed the mitochondrial DNA D-loop region of Japanese native chickens. These results indicate that Chinese and Korean chickens were derived from Southeast Asia. Following the domestication of red jungle fowl, a non-game type chicken was developed, and it spread to China. A game type chicken was developed in each area. Both non-game and game chickens formed the foundation of Japanese native chickens.

Among all the jungle fowl species, though all of them showed high genetic diversity with each other, but on comparative scale, *G. sonneratii* seems to be most distant, while *G. varius* to be most close to *G. gallus* and this relation ship was supported with high bootstrap values (70 – 100 %). Fumihito *et al.* (1994) also reported distinct distance between *G. varius* and *G. gallus*. Fumihito *et al.* (1996) also reported that all the four jungle fowl species might have branched out from the common stem almost simultaneously in the evolutionary time scale. Based on nucleotide diversity in

539 bp of D loop region, Munechika *et al.* (1997) found comparatively more closeness between *G. gallus* and *G. sonneratii* in comparison *G. varius* and *G. lafayattei*. Fu *et al.* (2001a) suggested that the red jungle fowl is near of kin to *G. lafayetei*, *G. sonneratii* and *G. varius* one by one in proper order. Though these reports do not provide any distinct trend regarding genetic relatedness among different jungle fowl species, but they consistently showed distinct distances between them and our observation was also in well alignment with these reports.

IIB. mt DNA polymorphism between *G. g. murghi*, Other *G. gallus* subspecies and *Gallus* species

Amplification of mitochondrial genes

The specific universal primers were designed to amplify 12S-rRNA as well as 16S-rRNA genes. These genes were amplified using genomic DNA extracted from meat as template. The 12S-rRNA specific universal primers have successfully amplified a ~ 440 bp fragment in Indian red jungle fowl. Similarly, 16S-rRNA specific universal primers have successfully amplified a ~ 590 bp fragment in Indian red jungle fowl.

Though the genes selected were mitochondrial genes, but the in general the genomic DNA extracted also have the mitochondrial DNA, hence the genomic DNA can very well be used as template to amplify the mitochondrial genes. Girish *et al.* (2004) and Sharma *et al.* (2005) have used genomic DNA extracted from meat tissue / blood to amplify the 12S-rRNA gene in various livestock species including poultry species.

Mitochondrial DNA sequence is highly conserved in different species of animals (Antoinette *et al.*, 1995). This has enabled designing of universal primers for mitochondrial genes, which can amplify corresponding fragments in wide variety of organisms including birds and insects (Prakash *et al.*, 2000). Using universal primers for PCR amplification obviate the requirement for an internal control, which is otherwise used to monitor the success of DNA amplification. As vertebrates contain about 1,000-10,000 copies of

mitochondrial DNA per cell (Shadel & Clayton, 1997); PCR assays based on its amplification are more sensitive in comparison to single or low copy nuclear DNA targets (Partis *et al.*, 2000). Since, quantity of PCR product relates to the copy number of target DNA sequence when a very small amount of DNA is used as a template (Chikuni *et al.*, 1994), high copy number of mitochondrial DNA ensures sufficiently high quantity of PCR product.

Nucleotide diversity

The cloned amplified products of 16S rRNA as well as 12S rRNA were sequenced. Sequencing confirmed the size of pertial 12S rRNA and 16S rRNA genes to be 448 bp (Accession Number DQ885561) and 610 bp size (Accession number DQ867016), respectively. Alignment of the comparable portion of 12S rRNA gene from other domestic fowl breeds, *G. gallus* subspecies and *Gallus* species totaled 452 nucleotides positions. The *G. g. murghi* showed very low nucleotide diversity (0.44 %) with *G. gallus* subspecies including *G. g. domesticus*, while comparatively much higher nucleotide diversity was observed between *G. g. murghi* and other jungle fowl species (< 3 %). The Jukes-Cantor genetic distances also showed similar trend as the estimates between *G. g. murghi* and other *G. gallus* subspecies including *G. g. domesticus* ranged from 0.005 to 0.007, however the genetic distances between *G. g. murghi* and other three jungle fowls were from 0.035 to 0.042. Similarly, alignment of the comparable portion of 16S rRNA gene from other domestic fowl breeds, G. gallus subspecies and *Gallus* species totaled 612 nucleotides positions. Between *G. g. murghi* and other subspecies i.e. *G. g. gallus*, *G. g. spadiceus* and *G. g. bankiva* including *G. g. domesticus*, very low nucleotide diversity (0.3 %)was observed, while other jungle fowls showed a divergence of ~ 2.9 % from *G. g. murghi*. The Jukes-Cantor genetic distances also reflected the similar trend, as these estimates between *G. g. murghi* and other *G. gallus* subspecies including *G. g. domesticus* were 0.000 to 0.003, whereas the genetic distances between *G. g. murghi* and other jungle fowls were 0.015 to 0.029.

The phylogenetic analysis by Neighbour Joining method (Concensus tree based on 100 bootstrap replications) yielded identical topology when applied to 12S rRNA as well as 16S rRNA data, hence the phylogenetic analysis was made on combined data (Fig 1). The *G. g. murghi* made one cluster along with domestic fowl breeds and other *G. gallus* subspecies with a good bootstrap support (> 60 %). Other jungle fowls made separate groups. These results showed that *G. g. murghi* is also very close to its other allies as well as the domestic chicken breeds. No report is available on the genetic similarity and phylogenic relationship of *G. g. murghi*, barring a report grom Gupta et al. (2005), who reported very high genetic similarity (> 99 %) between *G. g. murghi* and White Leghorn. However there are several reports on genetic similarity between Indian red jungle fowl and domestic chicken using different type of DNA markers. Very close genetic relatedness between Indian red jungle fowl (*G. g. murghi*) and domestic fowl was also revealed by other DNA markers such as randomly amplified polymorphic DNA, micro/mini satellite associated sequence amplification (MASA) and microsatellite markers (Tomar *et al.*, 2007).

Summary

Summary

A resource population comprising of 15 individuals of red jungle fowl and from each of the four chicken breeds i.e. White Leghorn, Kadaknath, Aseel and Red Cornish were made. A set of 22 di-nucleotide microsatellite markers i.e. $(CA)_n$ used in Poultry Biodiversity programme ((http://w3.tzv.de/aviandiv/ index.html) were used. These markers are universally distributed as possible throughout the chicken genome. Out of the total 22 microsatellite markers, 21 were polymorphic, while MCW 103 proved to be monomorphic in all the populations. At the 21 microsatellite loci all the populations were polymorphic except RC at MCW 222 and WL at MCW 216. Frequency of polymorphic population ranged from 0.80 to 1.00. Across the

population, total number of alleles ranged from 5 (at MCW 222 locus) to 21 (at LEI 192 locus). Average number of alleles per loci was 11.14. At a particular locus, number of alleles also varied between the populations. In RJF, number of alleles ranged from 2 (MCW 222, ADL 112) to 12 (MCW 34), In WL, from 2 (MCW 248, MCW 222) to 9 (MCW 330), in KN from 3 (MCW 284) to 14 (MCW 34), in AS, from 2 (MCW 98, MCW 81, MCW 78) to 14 (MCW 183) and in RC from 2(MVW 98, MCW 295, MCW 81, MCW 216) to 9 (MCW 34). Average number of alleles in RJF, WL, KN, AS and RC were 5.7, 4.8, 6.6, 5.7 and 4.6, respectively.

Observed as well as expected heterozygocity varied with the marker as well as between the populations. Average observed hetrozygocity, pooled over the loci ranged from 0.255 in WL to 0.384 in AS, while across all the populations, it was 0.346. Over all the loci, average heterozygocity ranged from 0.585 in RC to 0.760 in KN, whereas across the all populations, average heterozygocity was 0.835. Similarly the average PIC value ranged from 0.577 in AS and RC to 0.739 in KN, whereas across the all populations, it was 0.834.

A total of 234 alleles were score across the 5 populations and 21 microsatellite loci and 186 alleles were found in more than 1 population. For the five populations, 48 population specific alleles were identified, and majority of them i.e. 33 alleles were present at the frequency of 0.10 and more. Out of these 48 population specific alleles, 21 were specific to RJF, 9 were specific to WL, 6 were specific to KN, 10 were specific to AS and 2 were specific to RC. Among the 21 RJF specific alleles, 6 alleles at LEI 192 locus and 4 alleles at MCW 14 locus were more important from the RJF identification point of view as these alleles, are on extreme side of the allelic range. Considering all the chicken population together, there are 114 alleles, which are present in domesticated chicken, but absent in RJF.

Within population genetic similarity (WF) in different populations was estimated in terms of the band frequency. Based on the band frequency

across all the microsatellite loci, low within population genetic similarity was observed in all the population as the WF estimates were 0.289, 0.314, 0.217, 0.324 and 0.360 in RJF, WL, KN, AS and RC populations, respectively. These low levels of genetic similarity revealed that the population was quite variable at these microsatellite loci. Similarly, based on the band frequency across the all microsatellite loci, the genetic similarity estimates between the population ranged from 0.181 to 0.312. RJF showed low genetic similarity with the chicken breeds. At this low level of genetic similarity, WL seemed to be more close to RJF and RC seemed to most distant. Among the different chicken breeds, WL showed very low genetic similarity with AS and RC (0.182 – 0.185) in comparison to KN (0.312). Similarly RC showed more genetic similarity with KN and AS (0.256 – 0.298) in comparison to RJF and KN ().181 – 0.185). Both the native breeds showed more genetic similarity with each other (0.303) in comparison to other breeds. Similarly, pooled over the markers, the estimates of genetic distances between the populations ranged from 1.193 to 1.705 and reflected more or less similar trend.

Nucleotide sequence variation in two ribosomal genes (12S rRNA and 16S rRNA), two ATPase genes (ATPase 6 and ATPase 8), three Cytochrome Oxidase genes (COI, COII & COIII), one Cytochrome B gene (Cytb) and 7 NADP dehrdrogenase genes (ND1, ND2, ND3, ND4, ND4L, ND5 and ND6) was used to estimate genetic diversity between domestic fowl breeds (*G. g. domesticus*), three *G. gallus* subspecies (*G. g. spadicus*, *G. g. gallus* and *G. g. bankiva*) and three other *Gallus* species (*G. varius*, *G. lafayetei*, *G. sonneratii*). Complete mitochondrial DNA sequences from different individuals under the genus *Gallus* were retrieved from database. Sequences corresponding to the different genes were isolated and saved. Subsequently, the sequences were aligned using CLUSTALW and Jukes-Cantor genetic distances were estimated. Phylogenetic trees were constructed with neighbour joining (NJ) procedure. For most of the genes, except ribosomal genes, no size variation was observed between *G. gallus* subspecies and also between *Gallus* species. For 12S-rRNA, size variation was observed between

different *Gallus* species only, whereas between the *G. gallus* subspecies as well as between different breeds of domestic fowl, the size was conserved. However for 16S rRNA, size variation was evident between different domestic fowl breeds. Apart from size similarity, very high sequence homology was observed between the different domestic fowl for all the genes as the pair-wise genetic distances (Jukes-Cantor distances) ranged from 0.000 to 0.006. At this very high level of genetic similarity, the maximum nucleotide variability was limited to Tibetan native chicken and Silky. Similarly, between different *G. gallus* subspecies, very low nucleotide divergence was evident as the genetic distances between these subspecies ranged from 0.000 to 0.009. The different *Gallus* species showed comparatively high nucleotide divergence between themselves. The genetic distances ranged from 0.016 to 0.076. No definite trend between these *Gallus* species was evident for all the genes studied. However, *G. g. domesticus* showed distinctly and consistently very low nucleotide divergence with other *G. gallus* (0.000-0.009) in comparison to much higher nucleotide divergence (0.017-0.076) with other jungle fowl species for different genes.

Phylogenetic analysis made on cumulative nucleotide sequence variability for all the genes mentioned aboveshowed that different subspecies of *G. gallus* formed two clusters. While the one cluster includes exclusively *G. g. domesticus*, other cluster include *G. g. gallus*, *G. g. bankiva* and *G. g. spadicus* along with two *G. g. domesticus* breeds i.e. White leghorn and Silky. Rest of the three jungle fowls made separate group with very high bootstrap support (71-100). Similarly, the breeds within *G. g. domesticus* as well as other *G. gallus* subspecies clustered together.

Nucleotide sequence variation in two ribosomal genes (12S rRNA and 16S rRNA) used to estimate genetic diversity between Indian Red Jungle Fowl (G. gallus murghi), G. gallus subspecies (G. g. spadicus, G. g. gallus and G. g. bankiva) including *G. g. domesticus* (domestic fowl) and three other *Gallus* species (*G. varius*, *G. lafayetei*, *G. sonneratii*). Universal primers for mt 16S rRNA gene (Forward-5'- CGC CTG TTT ACC AAA ACA T-3', Reverse-5'- CCG

GTC TGA ACT CAG ATC ACG T-3') and for mt 12S rRNA gene (Forward-5'-CAA ACT GGG ATT AGA TAC CCC ACT AT-3', Reverse-5'-GAG GGT GAC GGG CGG TGT GT-3'), were used for PCR amplification. The 448 bp partial 12S-rRNA fragment (Accession Number DQ885561) and 610 bp partial 16S-rRNA fragment (Accession number DQ867016) were submitted to gene bank. The sequences of 12S rRNA and 16S rRNA genes from different domestic fowl breeds, other G. g. subspecies and Gallus species were retrieved from from the database. The 12S rRNA sequence (Accession Number DQ885561) and 16S-rRNA sequence (Accession number DQ867016) were aligned with the respective retrieved sequences and nucleotide diversity between the sequences was measured in terms of Jukes-Cantor distance. Phylogenetic trees were constructed with neighbour joining (NJ) procedure. The PCR amplification of the total genomic DNA of *G. g. murghi* (Indian red jungle fowl) using primers specific to 12S rRNA and 16S rRNA genes, amplified the partial sequences of 448 bp and 610 bp size, respectively. Alignment of the comparable portion of 12S rRNA gene from other domestic fowl breeds, *G. gallus* subspecies and *Gallus* species totaled 452 nucleotides positions. Between *G. g. murghi* and other subspecies i.e. *G. g. gallus*, *G. g. spadicus* and *G. g. bankiva* including *G. g. domesticus*, there was one insertion in and two nucleotide substitutions in *G. g. murghi*. However between *G. g. murghi* and other jungle fowl species, there were 3 - 6 insertions in jungle fowl species and 20 - 28 nucleotide substitutions. The *G. g. murghi* showed very low genetic distance with the domestic fowl breeds as well as with other *G. gallus* subspecies as the Jukes-Cantor genetic distance estimates ranged from 0.005 to 0.007, however the genetic distances between *G. g. murghi* and other three jungle fowls were high and ranged from 0.035 to 0.042. For 16 S RNA, very low nucleotide sequence variation was observed between the *G. gallus* subspecies, as except for two nucleotide substitutions in *G. g. murghi*, the sequences were similar. The Jukes-Cantor genetic distances also reflected the similar trend, as these estimates between *G. g. murghi* and other *G. gallus* subspecies including *G. g. domesticus* were 0.000 to 0.003.

However, G. *g. murghi* showed distinct difference from other jungle fowls as even at this very low level of genetic distances, the Jukes-Cantor distances between them were 0.015 to 0.029. The phylogenetic analysis by Neighbour Joining method (Concensus tree based on 100 bootstrap replications) yielded identical topology when applied to 12S rRNA as well as 16S rRNA data, hence the phylogenetic analysis was made on combined data. The G. *g. murghi* made one cluster along with domestic fowl breeds and other G. *gallus* subspecies with a good bootstrap support (> 60 %). Other jungle fowls made separate groups. These results showed that G. *g. murghi* is also very close to its other allies as well as the domestic chicken breeds.

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2 M Tris-HCl

Tris	242.2	g

Dissolve in 800 ml of autoclaved distilled water; adjust the pH 8.0 by HCl and make volume to 1000 ml. Sterilize by autoclave and store at 4°C.

0.5 M EDTA

EDTA	186.1	g

Dissolve in 500 ml of autoclaved distilled water with the help of magnetic stirrer for 1-2 hours and adjust the pH 8.0 by NaOH make volume to 1000 ml. Sterilize by autoclave and store at 4°C.

5M NaCl

NaCl	292.9	g

Dissolve in 800 ml of distilled water on magnetic stirrer and make volume up to 1000 ml. Autoclave and store at room temperature.

10% Sodium Dodecyl Sulphate

SDS	10	g
Autoclaved D.W.	100	ml

Store at room temperature and heat before use at 60°C.

3-M Sodium Acetate

Sodium Acetate	24.61	g

Dissolve in 100 ml D.W.
Autoclave it and store at room temperature.

Lysis Buffer (pH 8.0)

2 M Tris HCl (pH 8.0)	0.5	ml
0.5 M EDTA (pH 8.0)	0.2	ml
2 M NaCl	2.0	ml
10 % SDS	5.0	ml

Add autoclaved D.W. up to 100 ml and store at 4°C.

Tris Saturated Phenol

1. Melt phenol at 68°C by keeping on water bath.
2. Measure the required amount. Add 8-hydroxyquinoline at a final concentration of 0.1% (It is an antioxidant, gives yellow color to phenol).
3. Extract phenol several times with equal volume of 1 M Tris (pH 8.0).
4. Then with 0.1 M Tris, until the pH of the aqueous phase is more than 7.6.
5. Add 0.2 % β-mercaptoethanol.
6. Mix thoroughly and store in amber colored bottle at 4°C.

Chloroform-Isoamyl alcohol. (24:1)

Chloroform	24	ml
Isoamyl alcohol	1	ml

Mix thoroughly and store at 4°C.

Proteinase-K

Proteinase K	20	mg
Dissolve in 1 ml autoclaved D.W. Store at –20 °C		

Phenol- Chloroform-Isoamyl alcohol

Tris saturated phenol	25	ml
Chloroform- Isoamyl alcohol	25	ml
Mix thoroughly and store at 4 °C		

Tris-EDTA Buffer (T: E :: 10:1)

2M Tris-HCl	250	ml
0.5 M EDTA	100	µl
Autoclaved distilled water and store at 4°C.	50	ml.

20 X Tris Borate EDTA (TBE)

Tris base	216.0	g
Boric acid	110.0	g
0.5 M EDTA	80.0	ml
Autoclaved distilled water	1000	ml.
Sterilize by autoclave and store at room temperature.		

50 X Tris Acetate EDTA (TAE)

Tris base	24.2	gm
Glacial acetic acid	5.71	ml
0.4 M EDTA (pH 8.0)	25	ml
Autoclaved distilled water upto	1000	ml
Autoclave and store at room temperature.		

20000 X Ethidium Bromide (10mg/ml)

Ethidium bromide	10	mg
D. W.	1	ml
Store it in dark by wrapping the tube with aluminum foil.		

Loading Buffer

Bromophenol blue	0.25	%
Sucrose	40	%
Store at 4°C.		

Stop dye

formamide	80 %
50 mM Tris-Hcl (pH=8.8)	50 mM
EDTA	1mM
Bromophenol blue	0.1 %
Xylene cyanol	0.1 %